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SPIS TREŚCI

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CONTENT OF VARIOUS FORMS OF NITROGEN AND ABSORPTION OF THIS ELEMENT BY COCKSFOOT GRASS, DEPENDING ON METEOROLOGICAL CONDITIONS

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Abstract

The dependence of the content of various forms of nitrogen and absorption of N by cocksfoot grass on meteorological conditions was established in a field experiment. Correlations between the content of total nitrogen, protein nitrogen, $N\text{-}NH_4$ and $N\text{-}NO_3$ in sward and roots of cocksfoot grass and some meteorological elements, i.e. maximum temperature, minimum temperature, average daily temperature measured at 5 and 200 cm, air relative humidity, cloud cover, sum of precipitation, sum of evaporation and soil temperature measured at a depth of 2, 5, 10, 20 cm were analyzed. Correlation coefficients between the forms of nitrogen in the plant and the above meteorological elements were calculated, as well as multiple regression equations, multiple correlation coefficients and determination coefficients.

No significant relationship between the forms of N (N_{total}, $N_{protein}$, $N\text{-}NH_4$, $N\text{-}NO_3$) in sward and roots of cocksfoot grass and the course of meteorological conditions was clearly stated. The relationship between the content of $N\text{-}NH_4$ in sward and N_{total} and $N\text{-}NO_3$ in roots, and certain meteorological elements is relatively small and can be characterized by the value of determination coefficients, i.e. 0.166, 0.106 and 0.151, respectively. According to the statistical analysis, the absorption of nitrogen by cocksfoot grass depends to a relatively small though significant extent (R^2=0.249) on certain meteorological elements. However, further research is still recommended.

Key words: nitrogen forms, N uptake, cocksfoot grass, meteorological conditions.

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ZAWARTOŚĆ RÓŻNYCH FORM AZOTU ORAZ POBRANIE TEGO SKŁADNIKA PRZEZ KUPKÓWKĘ POSPOLITĄ W ZALEŻNOŚCI OD PRZEBIEGU WARUNKÓW METEOROLOGICZNYCH

Abstrakt

Na podstawie wyników ze ścisłego doświadczenia polowego określono zależność zawartości różnych form azotu oraz pobranie N przez kupkówkę pospolitą od przebiegu warunków meteorologicznych. Rozpatrywano korelacje między zawartością azotu ogółem, azotu białkowego, N-NH_4 i N-NO_3 w runi i korzeniach kupkówki a niektórymi elementami meteorologicznymi: temperaturą powietrza maksymalną, minimalną, średnią dobową mierzoną na wysokości 5 i 200 cm, wilgotnością względną powietrza, zachmurzeniem, sumą opadu atmosferycznego, sumą parowania i temperaturą gleby mierzoną na głębokości 2, 5, 10, 20 cm. Obliczono współczynniki korelacji między formami azotu w roślinie a przebiegiem wymienionych elementów meteorologicznych oraz równania regresji wielokrotnej, współczynniki korelacji wielokrotnej i determinacji.

Nie stwierdzono jednoznacznie, że istniała istotna zależność zawartości form azotu ($N_{og.}$, $N_{biał.}$, N-NH_4, N-NO_3) w runi i korzeniach kupkówki pospolitej od przebiegu warunków meteorologicznych. Zależność, która wystąpiła między zawartością N-NH_4 w runi oraz $N_{og.}$ i N-NO_3 w korzeniach a niektórymi elementami meteorologicznymi, jest stosunkowo niewielka i można ją scharakteryzować wielkością współczynników determinacji, kolejno: 0,166; 0,106 i 0,151. Pobranie azotu przez kupkówkę pospolitą zależało w stosunkowo niewielkim, lecz istotnym, stopniu (R^2=0,249) od przebiegu niektórych elementów meteorologicznych. Wskazują na to rezultaty obliczeń statystycznych; nieodzowne jest kontynuowanie prac dotyczących omawianych zagadnień.

Słowa kluczowe: formy azotu, pobranie N, kupkówka pospolita, warunki meteorologiczne.

INTRODUCTION

The chemical composition of plants depends on various factors. Above all, the content of organic and mineral compounds is influenced by agrotechnical factors (mainly mineral fertilization) and natural elements, e.g. soil quality, mainly its richness in available forms of nutrients, light, air and soil temperature, as well as precipitation (Clarkson and Warner 1979, Krzywy et al. 1985, Bednarek 2005, Bednarek et al. 2008, Wojciechowska 2004). The accumulation of protein, starch and dry matter in plants may significantly depend on certain meteorological elements (Solecka 1994, Kubik-Dobosz 1998, Ciepiela et al. 2001, Puła and Skowera 2004, Skowera et al. 2007).

Air temperature dropping to 5-6^{o}C and below favours starch conversions into soluble carbohydrates. Accordingly, the osmotic potential of cells increases and, as a consequence, the freezing resistance of plants rises. The sweetish flavour of frostbitten potato bulbs is also connected with these conversions. In contrast, a temperature increase within the range of 0-30^{o}C, with an optimal temperature of 20-30^{o}C, accelerates enzymatic reactions. Numerous complex processes related to the absorption and reduction of NO_3^- ions and the assimilation of NH_4^+ ions affect the content of nitrates in plants

(CLARKSON, WARNER 1979, HASEGAWA 1990, KUBIK-DOBOSZ 1998, WOJCIECHOWSKA 2004).

The aim of the research was to determine the relationships occurring between the content of various nitrogen forms in sward and roots of cocksfoot grass or the absorption of N by this plant and the local meteorological conditions.

MATERIAL AND METHODS

The paper is based on results obtained from a field experiment carried out on an experimental farm in Elizówka between 1987 and 1989. The experiment concerned the influence of fertilization with diversified doses of NPK on the yield and quality of cocksfoot grass. The content of macro- and microelements depends to a considerable degree on the richness of soil in nutrients as well as on the agrotechnical treatments. The presence of various forms of N (N_{total}, $N_{protein}$, N-NH_4, N-NO_3) in the aerial parts (sward) and roots of cocksfoot grass and the uptake of this element depended mainly on mineral fertilization, including N. This part of the experiment was presented in a previous article, which contains detailed information on the experiment and the chemical methods used in order to determine the examined forms of N in the plant (BEDNAREK 2005). The results of meteorological measurements and observations used here are decade average values, which include the period from March to October, and, in the authors' opinion, have the most significant influence on the chemical composition of the plants shown in Figures 1 and 2. The following factors were taken into consideration in the experiment: the maximum temperature marked in the figures as $T_{max.}$; minimum temperature $T_{min.}$; average daily temperature measured at 200 cm $T_{average\ at\ 200cm}$; average daily temperature measured at 5 cm $T_{average\ at\ 5\ cm}$; relative air humidity; cloud cover measured on a scale of 1-10; precipitation; evaporation; soil temperature measured at a depth of 2 cm, $T_{at\ depth\ 2\ cm}$; soil temperature measured at a depth of 5 cm $T_{at\ depth\ 5cm}$; soil temperature measured at a depth of 10 cm $T_{at\ depth\ 10\ cm}$; soil temperature measured at a depth of 20 cm $T_{at\ depth\ 20\ cm}$. The correlation coefficient was calculated between the determined forms of N and the meteorological conditions (the figures and the text include only the essential values). Additionally, multiple regression equations, multiple correlation coefficients (R), determination (R^2) and the significance level (p) were calculated. The dependent variables y were the content of the determined forms of N in sward and roots and N uptake by cocksfoot grass, respectively. The independent variables x were the meteorological elements in the region of the experiment (x_1 – air temperature measured at 5 cm, x_2 – air relative humidity, x_3 – cloud cover, x_4 – precipitation, x_5 – sum of evaporation, x_6 –

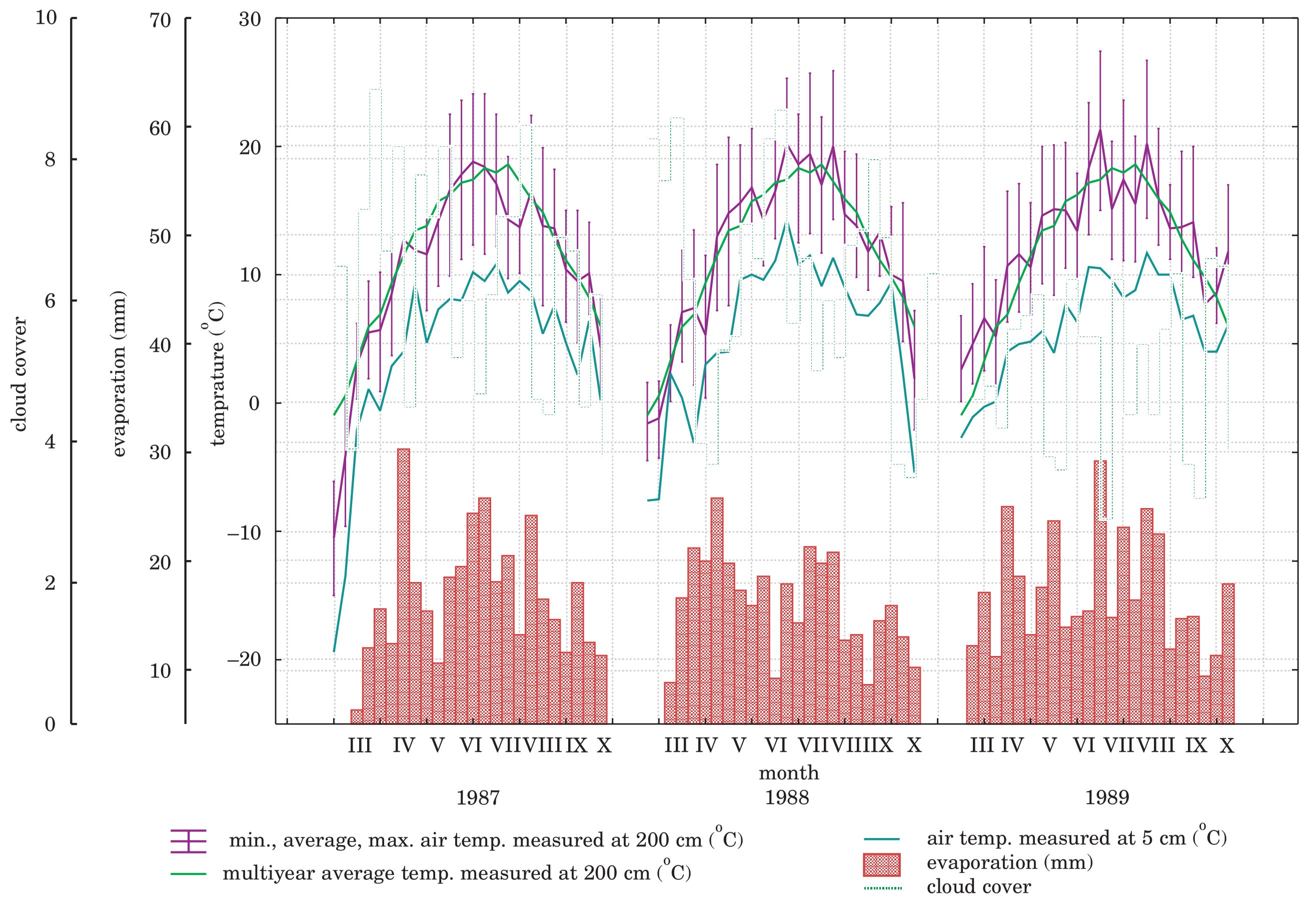

Fig. 1. Air temperature, precipitation and cloud cover in the area of the experiment

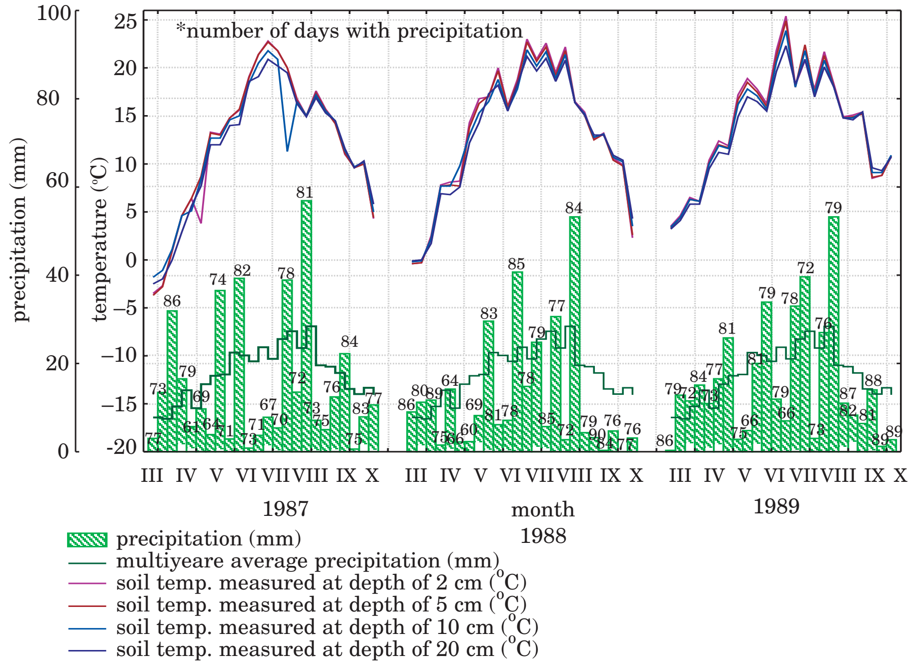

Fig. 2. Soil temperature, precipitation and the number of days with precipitation in the area of the experiment

soil temperature measured at 5 cm). In order to eliminate collinearity and on account of the high correlation between the variables, one of the four variables which characterise air temperature and one of the four variables which characterise soil temperature at various depths were chosen for further analysis. The calculations were made using Statistica ver. 6.0 and Statgraphics Plus 5.0.

RESULTS AND DISCUSSION

The yield and the chemical composition of plants are influenced not only by anthropogenic elements but also by meteorological conditions dominating in the region of the experiment (KUBIK-DOBOSZ 1998, PUŁA and SKOWERA 2004, WOJCIECHOWSKA 2004, SKOWERA et al. 2007, BEDNAREK et al. 2008).

The content of the forms of N in cocksfoot grass significantly depended on mineral fertilization, especially with nitrogen. In sward, the total content of N ranged from 1.76 to 2.84% in dry matter, and in roots – from 0.88 to 1.41% in dry matter. The content of protein nitrogen in sward ranged from 1.15 to 1.79% in dry matter, and in roots from 0.72 to 1.02% in dry matter.

The content of ammonium nitrogen in sward ranged from 0.11 to 0.27% in dry matter, and in roots from 0.10 to 0.21% in dry matter. The content of nitrate nitrogen (V) in sward ranged from 0.03 to 0.10% in dry matter, and in roots from 0.019 to 0.02% in dry matter (BEDNAREK 2005). The correlation coefficients between the meteorological elements shown in Figures 1 and 2 and the content of various forms of nitrogen in sward (aerial parts) and roots of cocksfoot grass indicates that the relationships were non-significant.

There was only one statistically proven positive correlation (r_{xy}=0.25; p=0.034; n=72), between cloud cover and content of ammonium nitrogen in sward. On the other hand, there is a significant negative correlation between N uptake by cocksfoot grass and air temperature (T_{max}., r_{xy}=-0.34; p=0.04; T_{min}., r_{xy}=-0.33; p=0.04; $T_{average}$, r_{xy}=-0.32; p=0.007; $T_{5\ cm}$, r_{xy}=-0.27; p=0.022) and soil ($T_{at\ depth\ of\ 2cm}$, r_{xy}=-0.34, p=0.004; $T_{at\ depth\ of\ 5\ cm}$, r_{xy}=-0.29, p=0.013; $T_{at\ depth\ of\ 10\ cm}$, r_{xy}=-0.30, p=0.009; $T_{at\ depth\ of\ 20\ cm}$, r_{xy}=-0.33, p=0.005) and the sum of evaporation (r_{xy}=-0.24, p=0.042), whereas for cloud cover there is a statistically proven positive correlation (r_{xy}=0.30, p=0.01) – Figures 3 and 4.

It seems, however, that multiple regression is a better measure of the relationship between the content of various nitrogen forms in plants and meteorological elements.

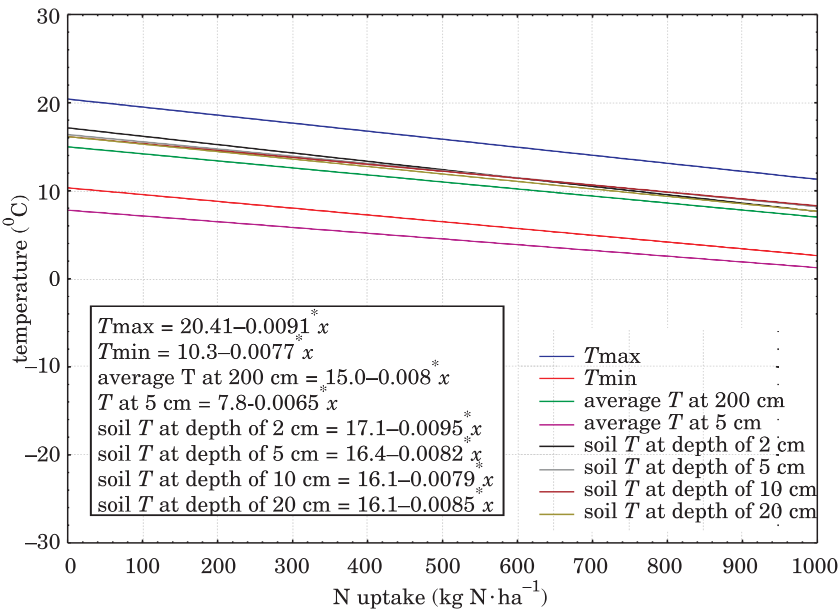

Fig. 3. Relationship between N uptake and air and soil temperature

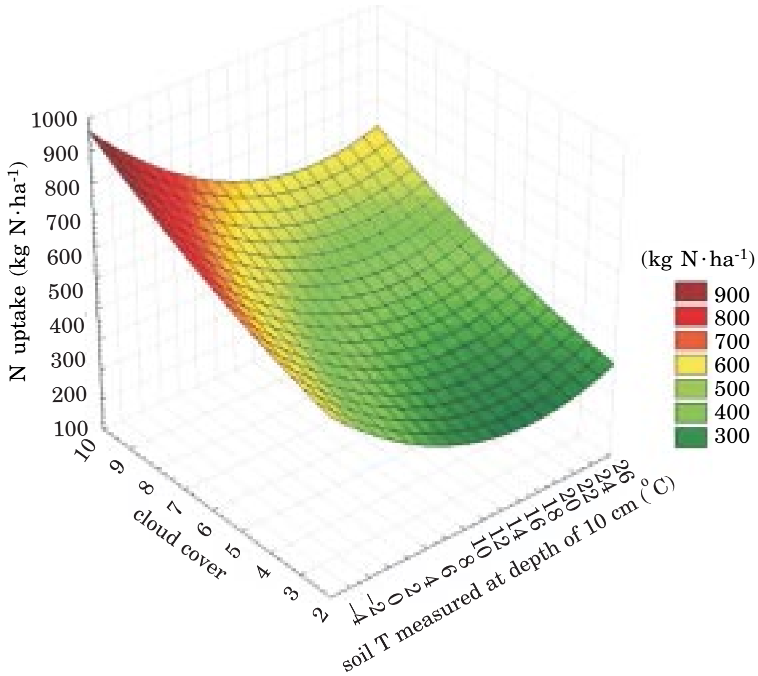

Fig. 4. Relationship between N uptake and cloud cover and average soil temperature measured at a depth of 10 cm

This calculation showed the content of NH_4 in sward to be significantly dependent on certain meteorological elements (R=0.407; R^2=0.166; p=0.0317). This relationship may be described by the following equation: $Y=0.487+0.019x_3-0.005x_2+0.005x_1-0.004x_5$; ($Y$ – the content of N-NH_4 in cocksfoot grass sward in % N in dry matter; notations of independent variables – see Methodology). However, it needs to be highlighted that certain meteorological elements (cloud cover, air relative humidity, $T_{5\ cm}$, evaporation) determined the content of NH_4 only in approx. 17%. The content of the remaining forms of nitrogen (N_{total}, $N_{protein.}$, N-NO_3) did not significantly depend on the examined meteorological elements. Puła and Skowera (2004) stated that a considerable amount of precipitation during the vegetation season of potato significantly limited the content of starch and dry matter, but favoured the accumulation of total protein in tubers. At the same time, the yields of starch, total protein and dry matter were significantly lower in the year receiving the largest amount of precipitation (1997). In other studies, Skowera et al. (2007) report that the content of total protein in chickpea seeds significantly depended on the sum of precipitation and the sums of effective temperatures in the ripening phase of pods.

The content of starch depended on the sum of precipitation in the bloom phase, the sums of effective temperatures in the phase of pod setting and the number of days with precipitation during the vegetation season. At the same time, CIEPIELA et al. (2001) reported that the length of the low temperature stress had influenced changes in the content of total and soluble protein in cv. Kargo spring triticale seedlings. Comparing the electrophoretic images of winter and spring wheat seedling proteins which have different frost resistance, SOLECKA (1994) stated that most of low temperature induced proteins were found in both types of plants. The only difference was the presence of a protein with a mass of 200 kDa in the winter variety, more resistant to low temperature. It suggests that most changes caused in cells by this factor are not connected with water freezing tolerance but with adjustment of the plant metabolism to low temperature. Analysis of protein distribution in leaves, nodes and roots of wheat showed that the 200 kDa protein is present in all tissues, but accumulates in much higher amounts in aerial tissues, which are much more frost-resistant than roots. In other words, the higher degree of synthesis of this protein is closely connected with the higher frost-resistance.

In addition, statistical calculations show that the content of total and nitrate nitrogen in roots of cocksfoot grass significantly depended on certain meteorological elements. The content of the former (N_{total}) can be described as follows: R=0.326; R^2=0.106; p=0.05. The multiple regression equation with the choice of the best subset of independent variables has the following form: $Y=0.128–0.184x_3+0.027x_2+0.027x_1$; (y – the content of N_{total} in roots in % N in dry matter; independent variables notations – see methodology). However, it needs to be highlighted that the independent variables x in the equation determined the content of total nitrogen in roots of cocksfoot grass only in approx. 11%. Moreover, statistical calculations show that there is some small but important relationship between the content of nitrate nitrogen in roots of cocksfoot grass and certain meteorological elements. It can be described in the following way: R=0.388; R^2=0.151; p=0.0107. The calculated multiple regression equation has the following form: $Y=0.034–0.0007x_1+0.0004x_4–0.0029x_3$; ($y$ – the content of N-NO_3 in roots in % N in dry matter; independent variables notations – see methodology). It needs to be underlined, however, that these meteorological elements determine the content of N-NO_3 in roots only in approx. 15%. KRZYWY et al. (1985) stated that the highest content of total nitrogen was found in sward gathered in the dry year 1983; nitrate nitrogen (V) in the control – also in dry years, and on the fertilizing objects – in the wet year.

The statistical calculations also showed that there is a particularly important relationship between N uptake by cocksfoot grass and some meteorological elements (Figures 3 and 4). It can be determined as follows: R=0.499; R^2=0.249; p=0.0017. Accordingly, the multiple regression equation has the following form: $Y=1635.9+50.2x_3+5.38x_1–16.0x_2–11.3x_5–9.33x_6$; (y – N uptake in kg·ha^{-1}; notations of independent variables – see methodology). However, it needs to be highlighted that the features taken into consideration in the equation determined N uptake by cocksfoot grass only in approx. 25%.

KUBIK-DOBOSZ (1998) stated that there is little evidence connecting NH_4^+ uptake by plants and the presence of light. Prolonged exposure to dark results in a decreased uptake of ammonium ions and especially the rate at which these ions enter cells. At the same time, there is little inhibition of their outflow. On the other hand, no influence of temperature changes on NH_4^+ ion uptake by *Lolium multiflorum* and *Lolium perenne* was reported (CLARKSON and WARNER 1979), and its increase from 10° to 35°C slightly stimulated the uptake of these ions by seedlings of *Ceratonia siliqua*. The uptake rate increased rapidly after a temperature increase to 40°C (KUBIK-DOBOSZ 1998). WOJCIECHOWSKA (2004), reports that, apart from agrotechnical factors, the intensity of light, water supply and temperature have an important influence on the accumulation of nitrates in plants. Low light intensity connected with low photosynthesis intensity influences the increase in the content of nitrates in plants. The main reason for this relationship is the inductive influence of light on the activity of nitrate reductase, which is the key enzyme in the metabolism of nitrates, and on the production of assimilates in the process of photosynthesis which act as acceptors of the reduced form of nitrogen. The content of nitrates in plants during the vegetation season is modified to a larger degree by water relations. Periods of drought during cultivation of plants causes an increase in the content of nitrates, mainly because their reduction is largely inhibited. Increased accumulation of nitrates in plants growing under negative water balance is connected with a rapid decrease in photosynthesis resulting from limited access to CO_2, which is caused by stomatal closure. The assimilation of nitrates by plants decreased under substantial limited water content in soil and lower air relative humidity. Lower soil temperature usually inhibits nitrate uptake. At a soil temperature of 16°C some rice varieties take up half the amount of NO_3^- ions absorbed at a temperature of 28°C (HASEGAWA 1990). Temperature influences metabolic processes; it affects the intensity of photosynthesis and respiration and the activity of enzymes responsible for the reduction of nitrates and the synthesis of organic nitrogen compounds. The content of nitrates in plants is influenced by the efficiency of numerous complex processes connected with the uptake and reduction of nitrate ions and the assimilation of ammonium ions.

CONCLUSIONS

1. No significant relationship between the forms of N (N_{total}, $N_{protein}$, $N\text{-}NH_4$, $N\text{-}NO_3$) in sward and roots of cocksfoot grass and the course of

meteorological conditions was clearly stated. The relationship between the content of N-NH_4 in sward and N_{total} and N-NO_3 in roots and certain meteorological elements is relatively slight and can be characterised by the determination coefficients: 0.166; 0.106 and 0.151.

2. The statistical analysis showed that the N uptake by cocksfoot grass depends to a relatively small though significant extent (R^2=0.249) on certain meteorological elements. However, further research is still recommended.

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THE INFLUENCE OF BLACK CURRANT (*RIBES NIGRUM)* SEED EXTRACT ON EFFECTIVENESS OF HUMAN CERULOPLASMIN IN Fe(II) IONS ELIMINATION

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Abstract

Ions of irons, especially ferrous ions may be harmful for living organisms, because they generate reactive oxygen species like $O_2^{\bullet -}$ or $^{\bullet}OH$. Probability of the risk rises especially in pathological conditions, in which high level of iron is observed. For this reason scientists try to establish new methods that can support organism in eliminating reactive ferrous ions.

Nowadays, attention focuses on substances present in plants, especially polyphenols, whose administration prevents oxidative damages in iron overloading.

This new approach requires some research on behavior of plant-derived compounds in human organism, within a system containing other biomolecules involved in iron metabolism. The aim of this study has been to investigate the influence of black currant (*Ribes nigrum)* seed extract, a source of polyphenols, on the activity of ceruloplasmin, an enzyme participating in Fe(II) elimination from blood plasma in human organism. Depletion of Fe(II) caused by ceruloplasmin isolated from healthy blood donors was compared to its decrease in a system containing both ceruloplasmin and the extract. The results have shown that addition of a particular amount of the extract elevates the effectiveness of ceruloplasmin in eliminating Fe(II) from the sample but only under physiological condition (pH 7.4; T 37°C). In a weak acidic solution, addition of the extract does not lead to any change in Fe(II) concentration.

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Key words: black currant (*Ribes nigrum)* seed extract, polyphenols, ceruloplasmin, ferroxidase activity, ferrous ions.

WPŁYW EKSTRAKTU Z PESTEK PORZECZKI CZARNEJ (*RIBUS NIGRUM*) NA SKUTECZNOŚĆ LUDZKIEJ CERULOPLAZMINY W ELIMINOWANIU Fe(II)

Abstrakt

Jony żelaza, szczególnie jony żelazawe Fe(II), ze względu na wysoki potencjał oksydoredukcyjny mają zdolność generowania wolnych rodników, takich jak $O_2^{\bullet-}$ i $^{\bullet}OH$. W pewnych stanach patologicznych, którym towarzyszy przeładowanie organizmu żelazem, ryzyko pojawienia się wolnych jonów Fe(II) inicjujących wiele procesów wolnorodnikowych jest bardzo prawdopodobne. W tym celu dąży się do opracowania terapii, które w chorobach żelazozależnych wspomogą naturalne mechanizmy usuwania reaktywnych jonów żelaza z organizmu. Ostatnio dużą uwagę poświęca się aktywnym substancjom obecnym w roślinach, szczególnie związkom polifenolowym, ze względu na ich silne właściwości chelatujące. Ten nowy kierunek badań wymaga jednak wnikliwej analizy, która wyjaśni mechanizm współdziałania substancji roślinnych z innymi biomolekułami zaangażowanymi w prawidłowy metabolizm żelaza w organizmie oraz rozstrzygnie o bezpieczeństwie ich stosowania. Celem badań było ustalenie wpływu ekstraktu z pestek porzeczki czarnej (*Ribus nigrum*), bogatego w związki polifenolowe, na aktywność ludzkiej ceruloplazminy, enzymu uczestniczącego w usuwaniu Fe(II) w organizmie ludzkim. Ubytek Fe(II) obserwowany w obecności ceruloplazminy porównywano z jego ubytkiem w mieszaninie ceruloplazmina-ekstrakt. Stwierdzono, że dodatek ekstraktu podnosi skuteczność ceruloplazminy w eliminowaniu Fe(II) ze środowiska. Istnieje więc przypuszczenie, że związki pochodzenia roślinnego takie jak polifenole mogą wspomagać naturalne mechanizmy eliminowania Fe(II) w organizmie.

Słowa kluczowe: ekstrakt z pestek porzeczki czarnej (*Ribus nigrum*), polifenole, ceruloplazmina, aktywność ferroksydazowa, jony żelazawe.

INTRODUCTION

Iron is an essential element in human organism, necessary for such basic processes as oxygen transport or cell respiration. This element is an important component of many enzymes and metaloproteins, which are involved in DNA synthesis, cholesterol metabolism and processes of detoxification. Despite beneficial effects of iron, there are some dangers connected with the presence of unbound iron ions in biological fluids or tissues. Ions of irons, especially ferrous ions, may generate reactive oxygen species (ROS) in Fenton, Haber-Weiss reactions or during the process of their non-enzymatic oxidation. The level of reactive iron in human organism is precisely regulated by ceruloplasmin (Roeser et al. 1970, Stocks et al. 1974) blue plasma copper protein, composed of three 42-45 kDa domains that are homologous to each other (Ortel et al. 1984) and similar to domains in factors V and VIII of coagulation cascade (Church et al. 1984). Ceruloplasmin catalyzes the oxidation of ferrous ions into less reactive ferric ions, subsequently binded by transport and storage proteins: apotransferrin and apoferritin (Ryden 1984). Ferroxidase activity of the protein, prevents organism from ROS generation catalyzed by free ferrous ions.

In some pathological condition related to disorders of iron absorption and metabolism, regulation mechanism may fail and lead to uncontrolled increase of iron concentration in human organism. It is observed during the course of a such diseases as aceruloplasminemia, atransferinemia, beta-talasemia, obesity, hypertension or diabetes type II (Powell 2002). In the case of patients suffering from iron overload diseases, the production of ROS, especially hydroxyl radical is highly probable (Sochaski et al. 2002, Mccullough, Bartfayb 2007). Free radicals can cause oxidative modification of biomolecules, mainly lipids peroxidation, protein injury, DNA damage and lead to dysfunction of many organs or cancerogenesis. Under these circumstances it is necessary to take up some precautions which can support organism in eliminating reactive ferrous ions and prevent their dangerous effects. Nowadays, patients with iron overload diseases are treated with bloodletting or chelating therapy using synthetic, hard-assimilate compounds (Dagg 1974). Invasiveness of these methods and number of side effects force us to search for safer therapies.

Recently, many researches have focused on active substances derived from plants and supplied to organism in daily diet. Fruit and vegetables are rich in vitamin C, tokoferols, karetonids and polyphenols, which act as antioxidants. This important antioxidant action of the compounds is based on their ability to sequester metal ions, therefore some researchers pay attention to their use in metal poisoning.

It was indicated that the administration of polyphenols like quercetin, rutin and silibin reduces the oxidation processes in iron overload diseases (Zhao et al. 2005, Zhang et al. 2006) resulting from strong chelating activity of these compounds towards ferrous ions as well as from the ability of polyphenols to eliminate free radicals generated by metal (Afanas'ev et al. 1989, Moran et al. 1997, Yoshino et al. 1998, Zhao et al. 1998, Mira et al. 2002). *In vitro,* radical scavenging and iron-chelating activities are also observed for plant extracts containing polyphenols, like black currant, (*Ribes nigrum*) rich in anthocyanins and flavonols (Lugasi, Hovari 2003, Benvenuti et al. 2004)

Pharmaceuticals enriched with some plant extracts might support traditional therapies in iron overload diseases, but their safe use should be preceded some laboratory tests checking their properties under physiological conditions and their cooperation with other biomolecules involved in iron metabolism. The present study was focused on the influence of black currant seed extract, rich in polyphenols, on the activity of ceruloplasmin, the enzyme participating in one of the most important processes of Fe(II) elimination in human organism.

MATERIALS AND METHODS

MATERIALS

Ceruloplasmin (Cp) was isolated from serum of healthy blood donors and subjected to the process of purification (Hilewicz-Grabska et al. 1988). In the first stage the protein was filtered through the column DEAE – Sephadex A – 25 using 0.2 M acetate buffer (pH 5.5) followed by precipitation with ammonium sulphate. The protein solution was then denaturated in chloroform-ethanol mixture (1:9 v/v). The protein pellet was reextracted and dialyzed to the 0.05 M phosphate buffer with addition of sodium chloride. Particular volume of 6 M protein solution: 10; 20; 30; 40 or 50 µl, was diluted in phosphate buffer (pH 7.4 or 6.0) to the final volume of the sample (525 µl). Different concentrations of Cp were tested (0.12; 0.24; 0.4; 0.5 and 0.61 µM) separately or in system including the extract.

Black currant seed extract (BcE) was obtained from seeds obtained as a waste product in the fruit industry. Seeds of black currant were dried, crumbled, degreased and threefold extracted using 80% aqueous ethanol. Dry mass obtained after evaporation was dissolved in 96% ethanol. The total content of phenolic compounds in the extract of seed was 340 +/- 20 mg/ /100 g d.m. and was determined by Folin-Ciocaltau method, using caffeic acid as a calibration standard (Roura et al. 2006). The BcE was diluted in phosphate buffer (pH 7.4 or 6.0) to the final concentration 166.67 mg d.m. l^{-1}. Particular volume of the extract: 10, 20, 40, 50, 100 or 150 µl was added to the main sample and diluted to 525 µl. Different concentrations of extract (3.18; 6.36; 9.54; 12.72; 16; 31.75; 47.62 mg d.m. l^{-1}) were tested separately or in system including ceruloplasmin.

METHODS

Reagents

Phosphate buffer pH 7.4 and pH 6.0; 3-2-Pyridyl-5,6-diphenyl-1,2,4-triazine-4,4-disulfonic acid sodium salt (ferrozine); histidine; ferrous-ammonium sulfate (Mohr's salt). The chromogen solution was made as follows: 0.0249 g of 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4,4-disulfonic acid sodium salt was dissolved in 25 ml of phosphate buffer (pH 7.4). The ferrous ions solution was made as follows: 0.0216 g $(NH_4)_2\ Fe\ (SO_4)_2 \cdot 6H_2O$ was dissolved in 250 ml of deionized water. Histidine solution was made as follows: 0.0170g of histidine was dissolved in 100 ml of deionized water. All the reagents were stored for a maximum of one week in a shaded place. Before every experiment, ferrous ions were added to the reaction mixture as a complex with histidine, in ratio 1:1 (v/v). Histidine protects ferrous ions against the change of oxidation state.

Ferrozine assay

The ability of Cp and BcE to eliminate ferrous ions was determined using ferrozine, a chromogen that forms a highly colored complex with ferrous ions (EREL 1998). Concentration of Fe(II) ions in the reaction mixture was always constant but concentration of ceruloplasmin and extract were changed. The incubation of Fe(II) ions with ceruloplasmin and/or extract was conducted at constant temperature 37°C and pH 7.4 for 1 minute to create conditions close to physiological ones. In the second stage of the method only pH was lowered to the value 6.0. After 1 minute of incubation ferrozine was added and the resulting colour was measured spectrophotometrically at 564 nm. The ability of the tested compounds to eliminate Fe(II) was estimated by comparing the value of absorbance of a sample without any biomolecules participating in iron transformation (blank sample) with absorbance of a sample containing different concentrations of ceruloplasmin and/or the extract. Results were expressed as a depletion of Fe(II) ions in μM according to the formula:

$$\Delta Fe(II)=(A_0-A_n)/27,9 \quad (1)$$

where:

A_0 – blank sample,

A_n – sample,

27,9 – molar coefficient of absorbancy (27 900 $mM^{-1}cm^{-1}$).

In every single experiment one concentration of ceruloplasmin and/or extract was prepared in constant volume of sample 0.525 ml. Ferrous ions mixture was added to the solution of ceruloplasmin and/or extract 1 ml of histidine. All the reagents were mixed together and incubated for 1 minute. After 1 minute incubation, 900 µl of ferrozine was added to the 100 µl of reaction mixture.

Statistical analysis

Statistical analysis was carried out using the Instat Sigma software. The results were compared by both ANOVA test and Student's T-test. Statistical significance was set at the level of p=0.05.

RESULTS AND DISCUSSION

Ribes nigrum belongs to the berries which are a rich source of bioactive compounds possessing important biological properties. It has been demonstrated that extracts and juices from *Ribes nigrum* have antiviral, anti-inflammatory, antitumor abilities (DECLUME 1989, YOKO et al. 2003, KONNO, OKUBO 2005). Studies of biochemical profiles of *Ribes nigrum* berries and black currant juices by High Performance Liquid Chromatography (HPLC) revealed abundance of polyphenols, mainly anthocyanins (NIELSEN et al. 2003) and flavonols such as quercetin, kaempferol and myrecitin (MIKKONEN et al. 2001). *Ribes nigrum* seeds, which are often a waste product from food industry, can be a source of many substances such as fatty acids: oleic, linoleic, stearidonic acids, α-tocopherol and carotenes (PICURIC-JOVANOVIC et al. 2002). Like in berries, different classes of phenolic compounds are also detected in seed content (LU, YEAP FOO 2003). Some research suggested that 70% of the antioxidant capacity of *Ribes nigrum* extract could be attributed to its phenolic compounds content.

Polyphenols have been known as compounds advantageous for human organism, preventing many illnesses such as heart diseases or cancer (STONER, MUKHTAR 1995, DUTHIE et al. 2000). Recent studies show they may also be used as therapeutics in treatment of some iron overload diseases (AFANAS'EV 1995, PIETRANGELO et al. 2002, ZHAO et al. 2005, ZHANG et al. 2006). The beneficial effect of polyphenols is based on their ability to inhibit oxidation processes catalyzed by ferrous ions, either through their chelation and/or the elimination of metal-induced ROS. There are suggestions that orally administered polyphenols individually or in varied combinations might enhance the efficacy of prevention of the oxidative damage caused by the excess of iron. Plant extracts which are condensed mixture of phenolic compounds can be used as therapeutics, however, it is vital to first determine the influence of their components on other mechanisms occurring in human organism and participating in the maintenance of the proper iron level. The present study tried to define the effect of *Ribes nigrum* seed extract on human ceruloplasmin, the enzyme involved in one of the most important mechanism of Fe(II) elimination.

The results suggest that increased concentration of Cp cause a proportionally higher depletion of Fe(II) in the reaction mixture (Figure 1). A similar effect was noticed for the BcE, which effectively eliminates Fe(II) from the environment in a dose-depend manner (Figure 2). The behavior of the extract is probably connected with the presence of polyphenols as its major component, although the influence of other substances like ascobic acid and tocophrols cannot be excluded.

In the first part of the experiment, the solutions of Cp and BcE were studied separately, whereas in the second part they were combined as a mixture to check their joint effect on Fe(II) elimination. Three concentrations of Cp were prepared (0.12; 0.40 and 0.61 μM) and each was submitted to the reaction with different concentration of the BcE.

The loss of Fe(II) was greater in the mixture containing both Cp and the BcE than in the solution of Cp only (Figures 3*a,b,c*), so it was stated that components of BcE cooperate with Cp in the process of eliminating of Fe(II). Supporting effect of the BcE on the activity of Cp was observed in all the tested concentrations of the enzyme, although the most noticeable re-

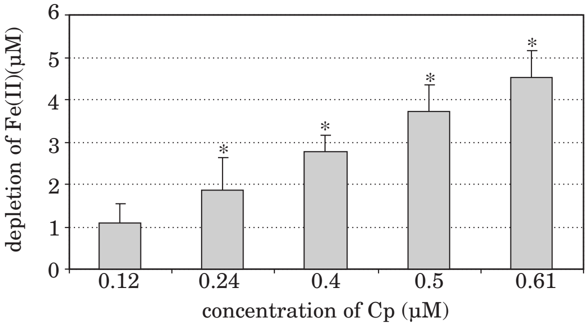

Fig. 1. Depletion of Fe(II) in the presence of different concentrations of Cp after 1 minute of incubation. Results were expressed as means ± SD. *$p \leq 0.05$ (concentration of Cp vs. the nearest neighbor concentration of Cp)

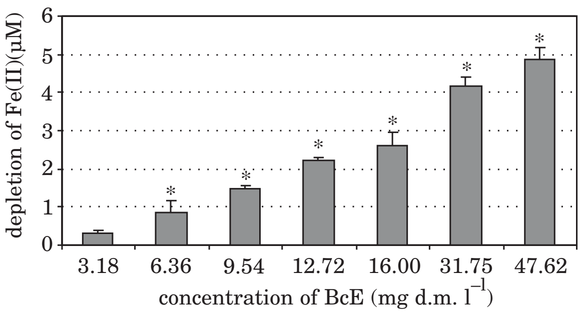

Fig. 2. Depletion of Fe(II) in the presence of different concentrations of BcE after 1 minute of incubation. Results were expressed as means ± SD. *$p \leq 0.05$ (concentration of BcE vs. the nearest, neighbor concentration of BcE)

sult appeared at the lowest Cp concentration (Figure 3*a*). Presumably, it is a consequence of the competition between Cp and the extract for the substrate in the reaction mixture. In a range of higher concentrations, Cp gains an advantage over the extract and reduces its participation in the process.

In the present study, all the experiments were conducted at physiological pH, but in some stage of the research the pH value of a reaction mixture was lowered to 6.0. Solutions of one concentration of Cp (0.61 µM) and BcE (12,72 mg d.m. l^{-1}) were chosen and tested separately and in mixture. A change of pH did not affect the effectiveness of Cp but dramatically lowered the extract involvement in Fe(II) elimination (Figure 4). For this reason, addition of the extract to Cp solution at pH 6.0 did not cause significant difference in the amount of eliminated Fe(II), observed previously at pH 7.4. This observation might be attributable to the influence of pH on the ability of polyphenols to form complexes with iron ions. This process has been described in earlier publications (MORAN et al. 1997, MIRA et al. 2002),

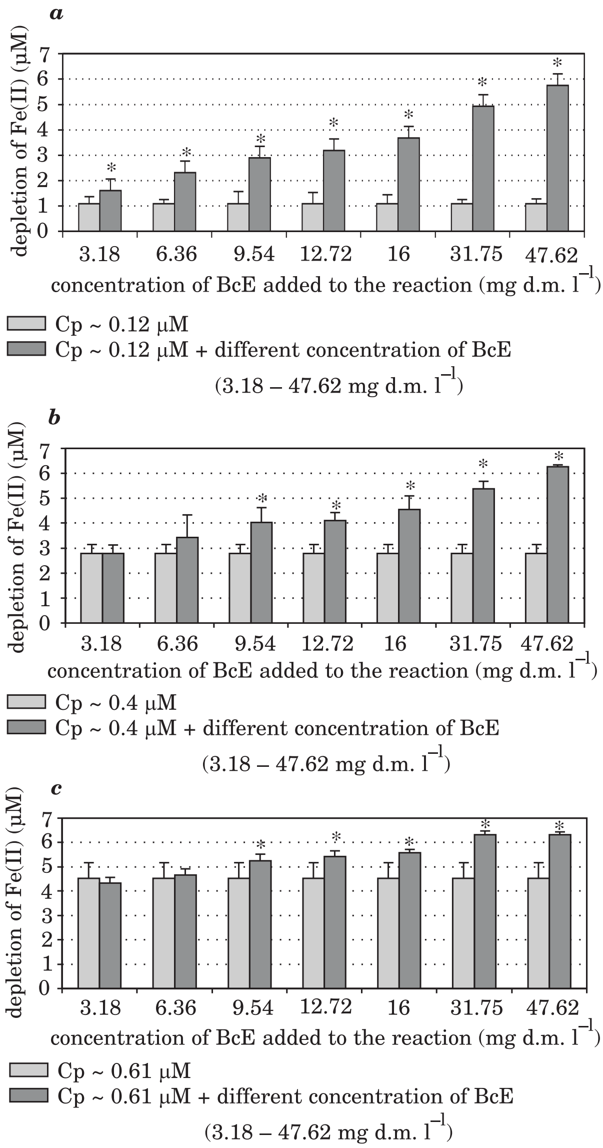

Fig. 3. Depletion of Fe(II) in the mixture containing constant concentration of Cp (a – 0.12 μM, b – 0.40 μM, c – 0.61 μM) and different concentrations of BcE (3.18-47,62 mg d.m. l^{-1}). Results were expressed as means ± SD.
*$p \leq 0.05$ (Cp vs. Cp + specified concentration of BcE)

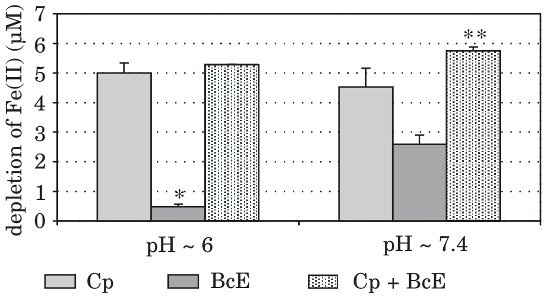

Fig. 4. Influence of pH on Cp, BcE and mixture containing both (Cp + BcE) in Fe(II) elimination. Results were expressed as means ± SD.
$p \leq 0.05$ (BcE at pH 6 vs. BcE at pH 7.4; **Cp at pH 7.4 vs. Cp + BcE at pH 7.4

in which the authors indicate that chelating properties of polyphenols in acidic pH are decreased .

Although our studies were carried out under *in vitro* conditions, they show that black currant extract rich in polyphenols raise the effectiveness of ceruloplasmin in Fe(II) elimination. The results could be more reliable if the natural processes occurring in human organism such as absorption and metabolism affecting natural abilities of nutritional compounds were taken into account. Apart from ceruloplasmin, it is necessary to focus on other endogenous antioxidants present in human organism, that could be either positively or negatively affected by active substances provided with food.

Plant extract are a mixture of different compounds, therefore it is difficult to analyse and determine the effect caused by individual substances. Iron elimination performed by BcE can result from the chelating activity of polyphenols or other compounds or their combination. Thus, this aspect must be carefully verified in the future studies.

Development of new approaches using plant compounds for treatment of iron overload diseases is a very important issue for future investigations concerning their safety, toxicity and their combined use with traditional medicines.

CONCLUSIONS

1. *Ribes nigrum* seed extract effectively eliminates Fe(II) from the environment in a dose-dependent manner. This effect may be connected with the presence of polyphenols as its major component. However, influences of other substances cannot be excluded.

2. The addition of *Ribes nigrum* seed extract elevates the effectiveness of ceruloplasmin in removal of Fe(II) from a sample provided it occurs under physiological conditions (pH 7.4; T 37°C).

3. *Ribes nigrum* seed extract rich in active polyphenols can support natural mechanisms regulating proper iron level, although this aspect should be confirmed by *in vivo* studies.

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INVESTIGATIONS OF Ni CONTENT IN HUMAN HAIR

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Abstract

Effect of nickel (Ni) on human organism is still evaluated, although there are few research papers dedicated to this problem. The participation of Ni in carcinogenesis and allergic reactions is considered. Nickel is absorbed through the alimentary tract, lungs and skin. Concentration of Ni in blood and urine is low (about 1 μg L^{-1}). More nickel has been determined in tissues such as liver, lungs and osseous tissue. The aim of this work was to assess the Ni level in human hair ($n = 220$, 110 women and 110 men) and correlations between Ni and other elements i.e. calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), lead (Pb), and cadmium (Cd). Hair washed with acetone and redistilled water was mineralized in mixture of HNO_3 and $HClO_4$ acids. Content of the elements was determined by the atomic absorption spectrometry method AAS. Concentrations of Ca, Mg, Zn, Cu, and Fe was measured by the flame technique (FAAS), while concentration of Pb, Cd and Ni was analyzed by the electrothermal atomic absorption spectrometry in a graphite furnace (GFAAS). The data were the subject of statistical analysis. The mean Ni concentration in the hair samples was 0.24 μg g^{-1} (median 0.17 μg g^{-1}, range 0.01-1.77 μg g^{-1}). Slightly more Ni was found in hair of women (0.25 μg g^{-1}) than of men (0.22 μg g^{-1}). Statistically higher concentrations of Ni were noticed in hair of men > 20 years than in younger men ($p<0.05$). Statistically significant positive correlations (for Zn, negative correlation) were established between Ni and Cd, Pb, and Cu concentration. Hair is available easily and non-invasively for tests and owing to a higher Ni level in hair than in physiological fluids, such tests help reduce analytical error. Therefore, hair is a very suitable material for monitoring elements in the human body.

Key words: nickel, bioelements, toxic metals, hair, atomic absorption spectrometry (AAS).

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BADANIA NAD ZAWARTOŚCIĄ NIKLU WE WŁOSACH LUDZKICH

Abstrakt

Oddziaływanie niklu (Ni) na organizm człowieka nadal jest przedmiotem badań, jednak liczba opublikowanych prac poświęconych tej tematyce jest niewielka. Rozpatrywany jest udział tego pierwiastka w procesie kancerogenezy oraz jako czynnika wywołującego alergię. Główne drogi wchłaniania Ni to przewód pokarmowy, płuca i skóra. Stężenie tego pierwiastka we krwi i moczu jest niskie i wynosi ok. 1 μg L^{-1}. Większe jego ilości oznaczono w tkankach (wątrobie, płucach, tkance kostnej). Celem pracy była ocena zawartości Ni we włosach (n=220, 110 kobiet i 110 mężczyzn) oraz korelacji między jego stężeniem a stężeniem innych pierwiastków: wapniem (Ca), magnezem (Mg), cynkiem (Zn), żelazem (Fe), ołowiem (Pb) i kadmem (Cd). Włosy umyte w acetonie i wodzie mineralizowano na mokro w mieszaninie kwasów HNO_3 i $HClO_4$. Zawartość pierwiastków oznaczano metodą spektrometrii absorpcji atomowej (AAS). Stężenie Ca, Mg, Zn, Cu i Fe wykonano techniką płomieniową (FAAS), natomiast Pb, Cd i Ni techniką bezpłomieniową w piecu grafitowym (GFAAS). Otrzymane dane poddano analizie statystycznej. Zawartość pierwiastków podano w zależności od płci i wieku. Średnia zawartość Ni w badanych próbkach włosów wynosiła 0,24 μg g^{-1} (mediana 0,17 μg g^{-1}, rozrzut 0,01-1,77 μg g^{-1}), nieco wiecej tego pierwiastka stwierdzono we włosach kobiet (0,25 μg g^{-1}) niż u mężczyzn (0,22 μg g^{-1}) i statystycznie więcej we włosach mężczyzn powyżej 20 lat w porównaniu z grupą wiekową poniżej 20. roku życia (p<0,05). Istotne statystycznie dodatnie korelacje (z wyjątkiem Zn) wyznaczono między zawartością Ni oraz Cd, Pb i Cu. Włosy są tkanką dostępną w sposób łatwy i nieinwazyjny, a ze względu na znacznie wyższą zawartość Ni w porównaniu z płynami ustrojowymi stanowią dobry materiał badań, co umożliwia zmniejszenie błędów pomiarowych podczas analizy.

Słowa kluczowe: nikiel, biopierwiastki, metale toksyczne, włosy, spektrometria absorpcji atomowej (AAS).

INTRODUCTION

Participation of nickel (Ni) in biochemical processes in the human organism remains the subject of research works. Nickel is absorbed by lungs, skin and the alimentary tract (with water and food). Fish, vegetables, corn, cacao and tea leaves contain high amounts of nickel (CHRISTENSEN 1995, BIEGO et al. 1998, KASPRZAK et al. 2003). In food, nickel may occur as a contaminant or can be added during technological processes. The daily dietary intake of Ni ranges between 170 and 400 μg, although it may be up to nearly 1 mg. Drugs containing Ni are an additional source of this element. The WHO (World Health Organization) recommends 100-300 μg Ni for daily intake (CORNELIS et al. 1995, IYENGAR 1998, KASPRZAK et al. 2003). Gastrointestinal absorption of Ni does not exceed 5% (an average 1-2%). About 80% of Ni is excreted mainly through the alimentary tract and kidneys (10 μg daily). Women retain 14% of consumed Ni, and men – 26%. High amounts of Ni have been found in the skeleton, lungs, skin, muscles, liver, and brain (an average 7,3 μg kg^{-1} body weight). The mean Ni concentration is the following: < 1-2 μg L^{-1} in serum, 1-5 μg L^{-1} in blood, and 17 ± 2 μg kg^{-1} in human milk (IYENGAR 1998, DENKHAUS, SALNIKOW 2002). In blood Ni is bound primarily to albumin, histidine and á-macroglobulin. It can also cross the placenta-blood barrier. Therefore, Ni concentrations in an adults and in a human fetus were found to be similar (CHRISTENSEN 1995). An amount of Ni absorbed via inhalation depends on particle size and solubility of its compounds. It passes through blood to be deposited in the pulmonary tract or eliminated. Citizens may inhale 0.2-1.0 μg of Ni per day. Cigarette smoking may contribute about 4 μg Ni/pack. The primary source of Ni compounds emission is industry related to fly-ash from burning fossil fuels, power plants, and road transport. Occupational exposure occurs e.g. in mining, welding, refining, alloy production, and electroplating. Soluble Ni compounds produced due to Ni-alloys corrosion may be the cause of contact dermatitis and local or systemic allergic reactions (CHRISTENSEN 1995, CORNELIS et al. 1995, KASPRZAK et al. 2003). Until present, no enzymes or cofactors containing Ni have been found in higher organisms. Such enzymes, however, have been identified in plant and bacterial cells (e.g. *Helicobacter pylori*) including urease, hydrogenase, CO-dehydrogenase, cis-trans isomerase, and Ni-superoxide dismutase. Thus, Ni may be indirectly required for normal functions of the digestive system. Animal experiments with Ni-deficient diet have demonstrated that Ni deficiency may be manifested as increased perinatal mortality, decreased growth and impaired iron (Fe) absorption from the intestine (anemia symptoms). Decreased activity of enzymes taking part in metabolism of carbohydrates, aminoacids and lipids (e.g. phospholipid synthesis) was also observed (DENKHAUS, SALNIKOW 2002). In humans, Ni toxicity may be a result of interactions of nickel with other elements, e.g. calcium (Ca), magnesium (Mg), zinc (Zn), manganese (Mn) and iron (Fe). Ni forms stronger bonds with ligands than bioelements i.e. Ca and Mg. Therefore, it can replace them in biologically active compounds (DENKHAUS, SALNIKOW 2002, KASPRZAK et al. 2003, SIDHU et al. 2004). In animals which were given $NiSO_2$ in drinking water the concentration of Zn, Cu, and selenium (Se) in liver significantly decreased, but concentration of Ni and Fe significantly increased. Zinc (as $ZnSO_4$) supplementation to these animals brought back the concentrations of elements to normal values. The authors suggest that Ni may promote excretion of elements from tissues. Increase in Fe concentration may be an implication of using the same absorption and transport mechanisms by both elements (Ni and Fe) (SIDHU et al. 2004). However, in animals which were given Fe-deficient diet, significantly increase in Ni concentration in serum, kidneys, skin, liver, lungs and testes was reported (TALLKVIST, TJÄLVE 1997). Increased Ni level was also noted in blood of patients with renal failure, rheumatoid arthritis, after acute myocardial infraction, in blood of patients taking some drugs and persons drinking wine or beer. The participation of Ni in allergic skin reactions is well documented now and it affects about 10% women and fewer men. This element is present in coins, jewellery, prosthesis and utensils. In 1990 IRAC (the International Agency for Research of Cancer) has concluded that Ni and its compounds are carcinogenic to humans. High amounts of Ni were found in the serum of patients with some types of cancer (e.g. lung and larynx), in the lung tissue of refinery workers and welders (even some hundred fold higher compared to the control group) and in breast cancer tissue (CHRISTENSEN 1995, CORNELIS et al. 1995, DENKHAUS, SALNIKOW 2002, KASPRZAK et al. 2003).

The Ni concentration in physiological fluids is low and may fluctuate during a 24-hour period. Therefore, we aimed to estimate the concentration of Ni in human hair depending on gender and age, and also the correlation between Ni and chosen elements (Ca, Mg, Zn, Cu, Fe, Pb, and Cd).

MATERIALS AND METHODS

Hair samples were taken from 220 persons (110 women and 110 men). They were cut at 6 different points of head (3-4 cm counting from the scalp) in amounts of about 0.5 g. The hair washed with redistilled water and acetone was mineralized in mixture of acids: HNO_3 and $HClO_4$. After mineralization, any excess of acids was removed, while the post-mineralization mixture was quantitatively transferred to volumetric flasks, which were filled up with deionized water (0.06 mS cm^{-1}).

Measurements were performed by the atomic absorption spectrometry method using an atomic absorption spectrometer AAS AVANTA Σ (GBC) equipped with an air-acetylene burner, graphite furnace GF3000 system with Ultra-Pulse background correction and autosampler PAL3000.

Concentrations of Ca, Mg, Zn, Cu, and Fe were determined by the flame technique FAAS under standard conditions, but concentrations of Pb, Cd, an Ni were measured by the flameless technique (GFAAS). The analyses were made in argon atmosphere as purging gas, in pyrolitic graphite tubes. The sample volume injected to the furnace was 20 μL. The peak area mode was used in calculations.

The instrumental parameters and analytical characterization of the methods are listed in Table 1. Table 2 presents results of the reference material analysis NCS 81002 (human hair). The data were verified by the standard addition method and analysis of the reference material.

Our results were expressed in terms of means and medians. The following methods were applied in statistical analyses: Shapiro-Wilks test of normality, Fisher test, *t*-Student test, Cochran-Cox, Kolmogorov-Smirnov test (for non-parametric distributions), Pearson test (for correlations). The level of statistical significance was assumed with $p<0.05$.

Table 1

Instrumental parameters and analytical characterization of the methods

Element	Wavelenght (nm)	Pyrolisis/ /atomization temperature (°C)	Calibration range (μg mL^{-1}) (ng mL^{-1})*	Limit of detection[3)] (μg mL^{-1}) (ng mL^{-1})*	Sensivity (μg mL^{-1}) (pg)*	Precison (%)
Ca	422.7		0.5-3.5	0.03	0.07	8.7
Mg	285.2		0.05-0.35	0.003	0.004	5.8
Zn	213.9		0.1-1.5	0.010	0.015	4.3
Cu	324.7		0.1-1.5	0.016	0.030	4.5
Fe	248.3		0.1-1.5	0.02	0.08	5.0
Pb[1)]	283.3	900/2000	1.5-15.0*	0.81*	5.8*	6.8
Cd[2)]	228.8	600/1800	0.15-1.50*	0.06*	0.36*	6.7
Ni	232.0	900/2400	1.5-15.0	0.66*	4.82*	8.7

Modifiers used: [1)]$NH_4H_2PO_4$,[2)]NH_4NO_3,[3)]Limit of detection (LOD) defined as 3SD (n = 10)
[4]Characteristic mass defined as amount of element giving an absorbance of 0.0044.

Table 2

Results of the certified material NCS ZC 81002 analysis, $x \pm s$, n = 6 (μg g^{-1})

Element	Certified value	Found value	Accuracy (%)
Ca	1090 ± 72	1051 ± 91	104.0
Mg	105 ± 6	103 ± 6	109.1
Zn	189 ± 8	185 ± 8	106.7
Cu	23.0 ± 1.4	22 ± 1.1	102.7
Fe	71.2 ± 6.6	65.2 ± 5.1	99.2
Pb	7.2 ± 0.7	7.4 ± 0.5	93.4
Cd	0.095 ± 0.012	0.105 ± 0.007	107.5

RESULTS AND DISSUSION

The content of Ni and the other examined elements in women's and men's hair is presented in Table 3. Concentrations of these elements in female hair depending on age (below and above the age of 20 years) are listed in Table 4, and in male hair – in Table 5. The mean Ni concentration in hair of 220 volunteers was 0.24 ± 0.24 μg g^{-1}, (median 0.17 μg g^{-1}, range 0.01-1.77 μg g^{-1}, percentiles (5%-95%) 0.04-0.74 μg g^{-1}). In women's hair slightly higher concentration of Ni was found than in men's (Table 3). In

Table 3

Concentration of Ni and other elements in hair of women and men (μg g^{-1})

Specification	Ca	Mg	Zn	Cu	Fe	Pb	Cd	Ni
N = 110	women							
Mean	454	19	182	20	12	0.6	0.07	0.25
Median	286	16	177	11	9	0.4	0.05	0.18
S.D.*	509	43	78	45	15	0.6	0.05	0.28
Range	51 3197	2 112	40 514	5 368	4 127	0.1 3.1	0.05 0.40	0.01 1.66
Percentyle 5%-95%	70 1378	5 43	63 303	7 42	5 26	0.1 1.7	0.05 0.17	0.03 0.74
N = 110	men							
Mean	291	18	159	12	11	1.2	0.13	0.22
Median	195	13	161	11	9	0.7	0.05	0.17
S.D.*	326	18	53	7	12	1.8	0.35	0.19
Range	16 1854	2 108	39 394	6 60	4 134	0.1 13.4	0.05 2.9	0.01 1.01
Percentyle 5%-95%	66 1139	3 60	64 244	7 20	5 17	0.2 3.4	0.05 0.42	0.05 0.62

* S.D. – standard deviation

Table 4

Concentration of Ni and other elements in hair of women depending on the age (μg g^{-1})

Specification	Ca	Mg	Zn	Cu	Fe	Pb	Cd	Ni
N = 56	women < 20 years							
Mean	407	17	169	17	13	0.8	0.07	0.27
Median	186	11	146	10	11	0.6	0.05	0.20
S.D.	523	18	93	38	12	0.6	0.04	0.29
Range	51 2523	2 112	52 514	7 296	5 92	0.1 2.8	0.05 0.23	0.02 1.66
Percentyle 5%-95%	64 1514	4 48	56 358	7 29	6 26	0.1 1.7	0.05 0.17	0.04 0.76
N = 110	men > 20 years							
Mean	503	22	195	24	12	0.5	0.08	0.23
Median	345	20	191	11	8	0.3	0.05	0.17
S.D.	494	13	55	51	17	0.6	0.07	0.26
Range	90 3197	7 91	40 328	5 368	4 127	0.1 3.1	0.05 0.40	0.01 1.63
Percentyle 5% - 95%	109 1378	8 41	72 276	7 94	4 33	0.1 1.7	0.05 0.17	0.02 0.53

women aged < 20 years the hair Ni concentration was higher than in older women (Table 4). In contrast, in hair adult men the Ni concentration was significantly higher in comparison with adolescent men ($p<0.05$) – Table 5. Due to a considerable variability range of the results, it seems reasonable to take medians as the best measure of the general tendency. Table 6 contains the Ni concentrations in human hair measured by other authors and proposed reference values for this element. It is concluded that Ni content in hair depends on sex, age, environmental and occupational exposition, nutritional habits, cigarette smoking and even hair colour. In the hair samples examined in the present experiment, the Ni concentration was significantly positively correlated with Cd, Pb, Fe and Cu concentration, but negatively correlated with Zn content. Similar observations, except Zn, are reported by SENOFONTE et al. (1989), NOWAK (1998), CHOJNACKA et al. (2005). Positive correlations may suggest that interactions between elements are synergistic, opposite to the negative direction, which indicates an antagonistic relationship. These interactions take place during absorption, transport, elimination, and cumulation processes. Positive correlations between Ni, Cd, and Pb may be a result of similar environmental exposition to heavy metals or smoking cigarettes. However, in the case of Fe, a possible explanation could be that Fe and Ni use the same absorption and transport processes, e.g. the divalent cation transporter system DMT-1. The negative direction of correlation between Ni and Zn concentrations confirms suggestions that Ni may both participate in Zn metabolism and impair the immune system under conditions excessive exposition to Ni.

Table 5

Concentration of Ni and other elements in hair of men depending on the age (μg g^{-1})

Specification	Ca	Mg	Zn	Cu	Fe	Pb	Cd	Ni
N = 63	women < 20 years							
Mean	248	12	147	13	12	0.9	0.07	0.17
Median	159	10	147	11	10	0.7	0.05	0.15
S.D.*	305	12	61	9	16	0.8	0.04	0.11
Range	16 1756	2 89	39 394	7 60	4 134	0.1 4.8	0.05 0.25	0.01 0.62
Percentyle 5%-95%	43 638	2 24	59 246	7 32	5 17	0.2 2.5	0.05 0.14	0.05 0.36
N = 110	men < 20 years							
Mean	395	26	177	11	9	1.5	0.23	0.29 $p<0.05$
Median	234	18	173	10	9	0.7	0.08	0.23
S.D.*	346	21	35	4	3	2.6	0.52	0.24
Range	66 1854	5 108	101 257	6 29	5 18	0.1 13.4	0.05 2.90	0.04 1.01
Percentyle 5%-95%	100 1205	7 68	129 236	7 17	6 14	0.2 4.4	0.05 0.60	0.06 0.87

Table 6

Content of Ni in hair according to other authors (μg g^{-1})

Number of samples	Arthmetic Mean	Median	Range	Country	Authors
106 95		0.6 1.3 (environmental exposition)	0.0 - 3.4 0.0 - 207.9	Czech Republic	BENCKO (1995)
44		1.17	0.45 - 12.45	India	SAMANTA et al. (2004)
92	0.868	0.349		Italy	VIOLANTE et al. (2000)
100	0.43	0.32	0.027 - 2.03	Italy	SENOFONTE et al. (1989)
266	0.75	0.30	0.03 - 10.0	Italy	SENOFONTE et al. (2000)
			0.02 - 0.20*	USA	IYENGAR (1998)
32		0.23	0.08 - 0.90*	France	GOULLE et al. (2005)
266	0.75	0.30		Poland	NOWAK (1998)
83	0.838			Poland	CHOJNACKA et al. (2005)

*Values reported as reference

Table 7

Pearson's correlation coefficient (r_{xy}) between concentration of Ni and other elements in hair

Specification	Ca	Mg	Zn	Cu	Fe	Pb	Cd
Women	-0.082	-0.124	-0.262 $p<0.01$	0.042	0.337 $p<0.001$	0.305 $p<0.00001$	0.498 $p<0.00001$
Women < 20	-0.134	-0.164	-0.243	-0.07	0.002	0.127	0.106
Women > 20	-0.001	-0.031	-0.284 $p<0.05$	0.155	0.606 $p<0.00001$	0.481 $p<0.001$	0.823 $p<0.00001$
Men	-0.005	0.123	-0.070	0.035	0.007	0.193 $p<0.05$	0.354 $p<0.001$
Men < 20	-0.009	0.079	-0.208	-0.048	0.001	0.435 $p<0.001$	0.414 $p<0.001$
Men > 20	-0.096	-0.018	-0.195	0.307 $p<0.05$	0.326 $p<0.05$	-0.094	0.332 $p<0.05$

CONCLUSIONS

1. Concentration of Ni expressed as a mean and a median found in the tested samples of hair is within the reference range.

2. Age and sex influence Ni concentration in hair. The mean Ni concentration in women's hair is higher in comparison to men's hair. Moreover, it is significantly higher in the group of men above 20 years of age.

3. The correlations between concentrations of Ni and other examined elements such as Ca, Mg, Zn, Cu, Fe, Pb, and Cd reflect their mutual interactions in biochemical processes (absorption, transport, cumulation, elimination). Positive correlations between Ni and Fe, Pb, and Cd can be linked with the synergistic character of these relations, but the negative correlation between Ni and Zn confirms antagonistic relations of both elements.

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MAGNESIUM FERTILIZATION OF SOIL CONTAMINATED WITH HEAVY METALS AND FORAGING OF SELECTED GNAWING PESTS

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Abstract

Magnesium fertilization of soil has been recommended as one of the ways to limit unfavourable effect of heavy metals on plants. Its effect may be connected with diminished heavy metal uptake by plants and changes in macroelement content. Therefore, the same measure may also change the host plant usability for potential herbivorous insects.

The paper contains compiled results of research on the effect of magnesium fertilization under conditions of soil contaminated with single heavy metals to level III of soil pollution according to the IUNG classification, on the foraging of *Bruchus rufimanus* Boh. and *Sitona* (*Sitona* sp.) on broad bean (*Vicia faba* L. ssp. *maior*).

It has been found that the applied fertilization level of soil contaminated with heavy metals does not affect significantly the yield of broad bean seeds, the degree of their damage due to *Bruchus rufimanus* or their germinating ability. Magnesium fertilization may slightly increase germinating energy of broad bean seeds from plants growing on cadmium contaminated soil. The effect of magnesium treatment under conditions of soil contamination with heavy metals on harmfulness of *Sitona* beetles to broad bean may be modified by atmospheric conditions in individual seasons. Magnesium fertilization of soil polluted with copper, lead, nickel and zinc to level III of soil pollution according to the IUNG classification does not lead to an increase in the degree of broad bean leaf damage by *Sitona* beetles. On the other hand, magnesium fertilization of soil contaminated with cadmium to level III of soil pollution in the IUNG classification may enhance broad bean plants' attractiveness to *Sitona*.

Key words: heavy metals, magnesium, *Sitona* sp. *Bruchus rufimanus* Boh.

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NAWOŻENIE MAGNEZOWE GLEBY SKAŻONEJ METALAMI CIĘŻKIMI A ŻEROWANIE WYBRANYCH SZKODNIKÓW GRYZĄCYCH

Abstrakt

Nawożenie magnezowe gleby jest polecane jako jeden ze sposobów na ograniczenie niekorzystnego oddziaływania metali ciężkich na rośliny. Może się to wiązać ze zmniejszeniem pobierania metali ciężkich przez rośliny i zmianami w zawartości makroskładników. Tym samym zabieg ten może również zmieniać przydatność rośliny żywicielskiej dla ewentualnych roślinożerców.

W pracy zestawiono wyniki badań nad wpływem nawożenia magnezowego w warunkach gleby zanieczyszczonej pojedynczymi metalami ciężkimi na poziomie III stopnia zanieczyszczenia wg klasyfikacji IUNG na żerowanie strąkowca bobowego (*Bruchus rufimanus* Boh.) oraz chrząszczy oprzędzików (*Sitona* sp.) na bobie (*Vicia faba* L., ssp. *maior*).

Stwierdzono, że zastosowany poziom nawożenia magnezowego gleby skażonej pojedynczymi metalami ciężkimi nie wpływa istotnie na plon nasion bobu, stopień ich uszkodzenia przez strąkowca bobowego ani też ich zdolność kiełkowania. Nawożenie magnezowe może nieco zwiększać energię kiełkowania nasion bobu pochodzących z roślin rosnących w glebie zanieczyszczonej kadmem. Wpływ nawożenia magnezowego w warunkach skażenia gleby metalami ciężkimi na szkodliwość chrząszczy oprzędzików dla bobu może być modyfikowany przez warunki atmosferyczne w danym sezonie. Nawożenie magnezowe gleby zanieczyszczonej miedzią, ołowiem, niklem i cynkiem na poziomie III stopnia zanieczyszczenia wg klasyfikacji IUNG nie powoduje wzrostu stopnia uszkodzenia liści bobu przez chrząszcze oprzędzików. Nawożenie magnezowe gleby zanieczyszczonej kadmem na poziomie III stopnia zanieczyszczenia wg klasyfikacji IUNG może natomiast przyczyniać się do wzrostu atrakcyjności roślin bobu dla oprzędzików.

Słowa kluczowe: metale ciężkie, magnez, *Sitona* sp. *Bruchus rufimanus* Boh.

INTRODUCTION

Magnesium fertilization of soils contaminated with heavy metals is recommended as one of the measures for reducing the uptake of these metals by plants (CURZYDŁO 1988). It may also improve the condition and growth of plants growing on polluted soil (JAWORSKA, GOSPODAREK 2003). Typically, gnawing insects less eagerly attack plants from polluted areas (BOCZEK, SZLENDAK 1992). Therefore, improvement of plant growth as a result of magnesium treatment may enhance attractiveness of these plants to this pest group.

The goal of the paper has been to compile the results of investigations on the effect of magnesium fertilization of soil contaminated with single heavy metals to level III of pollution according to the IUNG classification on harmfulness of *Sitona* sp. and bean beetle *Bruchus rufimanus* Boh. to broad bean *Vicia faba* L., ssp. *maior*.

MATERIAL AND METHODS

The experiments were conducted in field conditions in Zagaje Stradowskie, a village in Świętokrzyskie Province. Observations were conducted on broad bean (*Vicia faba* L., ssp. *maior)*, cv. White Windsor, cultivated on the following objects: unpolluted soil with natural heavy metal content (Control); unpolluted soil with natural heavy metal content receiving mineral fertilization (control+NPK); soil polluted with 4 $mg \cdot kg^{-1}$ d.m. of cadmium; soil polluted with a dose of 530 $mg \cdot kg^{-1}$ d.m. of lead; soil polluted with copper dosed at 85 $mg \cdot kg^{-1}$ d.m.; soil contaminated with a dose of 1.000 $mg \cdot kg^{-1}$ d.m. of zinc and soil polluted with 110 $mg \cdot kg^{-1}$ d.m. of nickel. The established level of soil pollution corresponded to a moderate pollution level in the IUNG classification. Identical magnesium fertilization was applied to all the objects: 20.4 mg $Mg \cdot kg^{-1}$ d.m.

Harmfulness of bean beetle was assessed on the basis of the weight of damaged seeds in relation to total seed mass. The assessment of broad bean seed germination energy and ability was conducted in laboratory conditions, following the generally accepted standards.

Harmfulness of *Sitona* sp. beetles was determined by measuring the leaf area loss, the consumed area and by counting injured and non-injured leaves. The damage was analysed twice (on 21.05.05 and 2.06.05). The significance of differences between means was established by means of one-way ANOVA. The means were differentiated by Duncan test at the significance level p=0.05.

Plant samples for chemical analyses were collected at the seed milk maturity stage. Because the experiment also assessed harmfulness of *Aphis fabae* Scop. aphid, which is the most serious broad bean pest, the sampling date was set to match its feeding period. Chemical analysis of the plant material comprised determining the content of heavy metals (cadmium, lead, zinc, copper and nickel). Detailed description of the methods applied was presented in other papers (GOSPODAREK, NADGÓRSKA-SOCHA 2007, GOSPODAREK 2008a).

RESULTS AND DISCUSSION

The applied magnesium fertilization caused 2-5 fold decline in the content of the analysed heavy metals in broad bean underground parts (Table 1). On the other hand, in the aerial parts a reduction of the metal content in resulting from the application of magnesium fertilization occurred in the case of zinc (Table 2). A similar effect of magnesium fertilization in relation to this element was noted in Italian ryegrass (GORLACH et al. 1980).

Table 1

The mean content of heavy metals in dry mass of roots of broad bean cultivated on soil with natural heavy metal content, soil contaminated with heavy metals and after application magnesium treatment (% in relation to control + NPK)

Objects	Content of heavy metals (% in relation to control + NPK)				
	Cd	Cu	Pb	Zn	Ni
Control	109 *a*	151 *a*	81 *a*	134 *a*	164 *a*
Control+Mg	46 *a*	62 *a*	79 *a*	114 *a*	166 *a*
Control+NPK	100 *a*	100 *a*	100 *a*	100 *a*	100 *a*
Control+NPK+Mg	41 *a*	51 *a*	72 *a*	101 *a*	420 *a*
Cd	1838 c	-	-	-	-
Cd+Mg	705 *b*	-	-	-	-
Cu	-	1890 c	-	-	-
Cu+Mg	-	485 *b*	-	-	-
Pb	-	-	3397 c	-	-
Pb+Mg	-	-	1005 *b*	-	-
Zn	-	-	-	46766 *c*	-
Zn+Mg	-	-	-	23831 *b*	-
Ni	-	-	-	-	16810 *c*
Ni+Mg	-	-	-	-	3362 *b*

Values in columns marked with different letters are statistically different at $p = 0.05$

Broad bean cultivated on soil contaminated by an aggregated dose of the analysed heavy metals, at an elevated content level in the IUNG classification, was found to have a reduced uptake of Pb, Cd and Ni by roots and Zn uptake by roots, leaves and pods under magnesium fertilization combined with liming (JAWORSKA, GOSPODAREK 2005). In the case of copper and cadmium, magnesium treatment did not significantly affect the content of these elements in broad bean aerial parts, whereas for lead and nickel a slight increase in their content was observed on contaminated soil subjected to magnesium fertilization (GOSPODAREK, NADGÓRSKA-SOCHA 2007) – Table 2. In the author's former investigations, reduced content of cadmium and nickel in broad bean pods was noted under the influence of magnesium fertilization applied to soil contaminated with an aggregate dose of heavy metals (Cu, Cd, Ni, Zn and Pb) to the level of elevated content (JAWORSKA, GOSPODAREK 2005).

Magnesium fertilization of soil contaminated with the analysed elements did not affect significantly the degree of leaf injuries due to *Sitona* sp. at an early stage of the plant development (1st date – 2nd decade of May) – Table 3, Figure 1 (GOSPODAREK 2008b). However, later (2nd date – 1st decade

Table 2

The mean content of heavy metals in dry mass of aerial plant parts of broad bean cultivated on soil with natural heavy metal content, soil contaminated with heavy metals and after application magnesium treatment (% in relation to control + NPK)

Objects	Content of heavy metals (% in relation to control + NPK)				
	Cd	Cu	Pb	Zn	Ni
Control	148 *b*	91 b	117 *b*	96 *a*	129 *a*
Control+Mg	58 *a*	131 *c*	98 *b*	106 *a*	138 *a*
Control+NPK	100 *b*	100 *b*	100 *b*	100 *a*	100 *a*
Control+NPK+Mg	53 *a*	72 *a*	98 *b*	91 *a*	104 *a*
Cd	438 *c*	-	-	-	-
Cd+Mg	453 *c*	-	-	-	-
Cu	-	226 *d*	-	-	-
Cu+Mg	-	215 *d*	-	-	-
Pb	-	-	1310 *d*	-	-
Pb+Mg	-	-	1960 *e*	-	-
Zn	-	-	-	24263 *c*	-
Zn+Mg	-	-	-	19104 *b*	-
Ni	-	-	-	-	11798 *b*
Ni+Mg	-	-	-	-	13138 *c*

Values in columns marked with different letters are statistically different at $p = 0.05$

of June), under the conditions of cadmium contaminated soil, a slight increase in the area damaged by *Sitona* sp. was noted following magnesium treatment (Table 3). Also, per cent loss of leaf blade was slightly higher in this object (Figure 1). No considerable effect of soil magnesium treatment on the degree of broad bean plant damage due to this pest occurred on soil contaminated with copper, lead and nickel. Plants growing on soil contaminated with zinc and subjected to magnesium fertilization were characterized by a considerably worse growth but were not injured by *Sitona* beetles. In the former research, magnesium fertilization of soil with elevated levels of heavy metals applied jointly did not cause any increase in the degree of broad bean damage caused by *Sitona* beetles (JAWORSKA, GOSPODAREK 2003).

Magnesium fertilization of soil contaminated with cadmium, copper and lead did not affect significantly the seed yield or the degree of seed injuries due to bean beetle (Table 4). The seed germination energy test revealed beneficial effect of magnesium treatment under the conditions of cadmium contaminated soil – Table 4 (GOSPODAREK 2008a). Plants growing on soil contaminated with zinc and nickel did not form seeds.

Table 3

Injuries caused by Sitona beetles on broad bean growing on natural soil (control, control + NPK), soil polluted with individual heavy metals and after application magnesium treatment (% in relation to control + NPK)

Objects	Leaves injured by *Sitona* sp. (% in relation to control+ +NPK) First date	Leaves injured by *Sitona* sp. (% in relation to control+ +NPK) Second date	Total consumed area per plant (% in relation to control+ +NPK) First date	Total consumed area per plant (% in relation to control+ +NPK) Second date
Cd	123 *a*	112 *e-g*	265 *a*	79 *c-e*
Cd+Mg	190 *a*	125 *g*	258 *a*	191 *g*
Cu	101 *a*	95 *d-g*	200 *a*	66 *a-e*
Cu+Mg	31 *a*	100 *d-g*	61 *a*	54 *a-d*
Control	90 *a*	114 *e-g*	229 *a*	88 *c-f*
Control+Mg	0 *a*	61 *bc*	0 *a*	52 *a-d*
Ni	*81 a*	99 *d-g*	145 *a*	30 a-d
Ni+Mg	16 *a*	94 *d-g*	58 *a*	47 *a-d*
Control+NPK	100 *a*	100 *d-g*	100 *a*	100 *d-f*
Control+NPK+Mg	19 *a*	80 *b-e*	26 *a*	76 *c-e*
Pb	103 *a*	109 *e-g*	113 *a*	125 *e-f*
Pb+Mg	16 *a*	121 *g*	3 *a*	87 *c-f*
Zn	0 *a*	52 *b*	0 *a*	6 *ab*
Zn+Mg	0 *a*	0 *a*	0 *a*	0 *a*

Means in columns marked with the same letter do not differ at $p = 0.055$

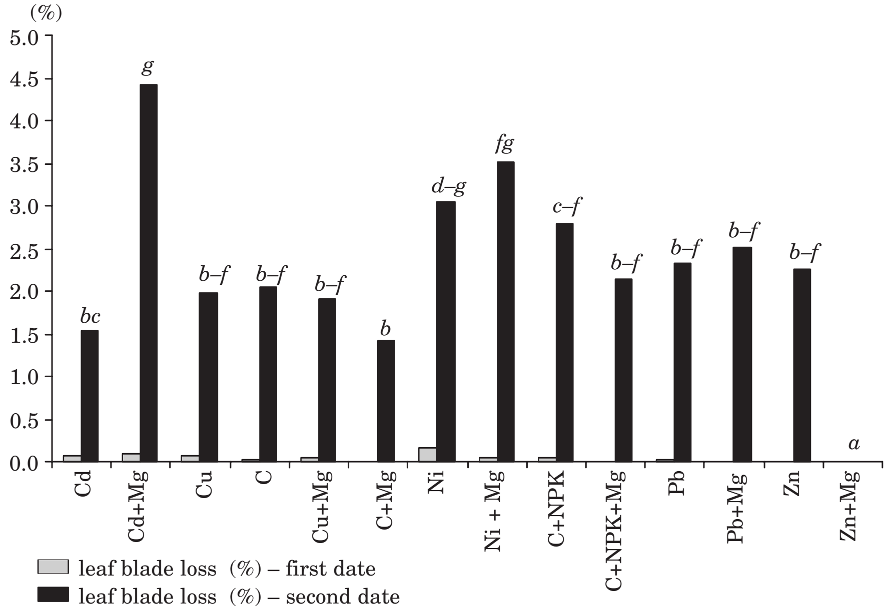

Fig. 1. Leaf blade loss due to *Sitona* beetle feeding (% of total leaf area) in broad bean growing on natural soil (control – C, control + NPK), soil polluted with individual heavy metals and after application of magnesium fertilization. Means marked with the same letter do not differ at p = 0,05. Assessments were presented only when statistical differentiation between means existed

Table 4

Injuries caused by *Bruchus rufimanus* Boh. beetles on broad bean growing on natural soil (control, control+NPK), on soil polluted with individual heavy metals and after application magnesium treatment. Germination energy and ability of broad bean seeds

Objects	Average seed weight per plant (g)	Seeds injured by *Bruchus rufimanus* Boh. (%)	Germination energy (%)		Germination ability (%)	
			uninjured seeds	injured seeds	uninjured seeds	injured seeds
Cd	7.501 *ab*	63.50 *ab*	0.011 *a*	0.000 *a*	81.02 *a*	58.03 *ab*
Cd+Mg	8.210 *ab*	72.31 *ab*	20.03 *b*	33.31 *b*	73.31 *a*	60.05 *ab*
Cu	6.340 *ab*	61.40 *ab*	6.732 *ab*	13.42 *ab*	93.31 *a*	46.71 *ab*
Cu+Mg	4.733 *ab*	76.32 *ab*	0.000 *a*	0.000 *a*	87.54 *a*	60.03 *ab*
Control	6.215 *ab*	89.53 *ab*	0.000 *a*	0.000 *a*	89.02 *a*	60.04 *ab*
Control+Mg	*2.803 a*	86.04 *ab*	0.000 *a*	0.000 *a*	72.01 *a*	45.01 *a*
Control+ +NPK	5.421 *ab*	55.53 *a*	0.000 *a*	0.000 *a*	87.03 *a*	47.02 *ab*
Control+ +NPK+Mg	6.324 *ab*	90.51 *ab*	20.52 *b*	6.712 *ab*	85.04 *a*	73.10 *b*
Pb	7.501 *ab*	92.83 *ab*	0.000 *a*	20.03 *ab*	75.02 *a*	73.32 *b*
Pb+Mg	9.323 *b*	98.12 *b*	0.000 *a*	13.31 *ab*	81.06 *a*	51.71 *ab*

Means in columns marked with the same letter do not differ at $p = 0.055$

CONCLUSIONS

1. Magnesium fertilization of soil contaminated with copper, lead, nickel and zinc to pollution level III according to the IUNG classification does not cause any increase in the degree of broad bean injuries by gnawing insects, such as *Sitona* and bean beetle.

2. Magnesium treatment may have a beneficial effect, increasing broad bean seed germination from plants growing on soil contaminated with cadmium.

3. Magnesium fertilization of soil contaminated with cadmium to pollution level III in the IUNG classification may contribute to improved attractiveness of broad bean plants for *Sitona* beetles.

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EFFECT OF CULTIVATION FACTORS ON MAGNESSIUM CONTENT IN AND REMOVAL BY THE POTATO TUBER CROP

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Abstract

Studies were carried out on samples obtained from a field experiment conducted on light loamy soil in 2002-2004. The experiment was set up as randomized sub-blocks with three replications. Two methods of soil tillage, conventional and simplified, were compared as well as seven weed control methods including application of herbicides or their mixtures: Plateen 41,5 WG, Plateen 41,5 WG + Fusilade Forte 150 EC, Plateen 41,5 WG + Fusilade Forte 150 EC + adjuvant Atpolan 80 EC, Barox 460 SL, Barox 460 SL + Fusilade Forte 150 EC, Barox 460 SL + Fusilade Forte 150 EC + adjuvant Atpolan 80 EC and control object (mechanical weeding). Magnesium content and its removal by potato tuber crop significantly depended on soil tillage methods, weed control methods and weather conditions over the growing season. More magnesium was contained in tubers harvested from the plots where reduced tillage had been applied (1.771 g kg^{-1} on average), compared with 1.762 g kg^{-1} under the conventional method. Herbicides and their mixtures applied to control weeds in the potato field significantly increased magnesium content in tubers and its removal by tuber yield by an average 2.7% and 11.0%, respectively, compared with the control where weeds were controlled by means of mechanical cultivation.

Key words: potato tubers, magnesium, content, uptake.

WPŁYW ZABIEGÓW AGROTECHNICZNYCH NA ZAWARTOŚĆ I POBRANIE MAGNEZU Z PLONEM BULW ZIEMNIAKA

Abstrakt

Badania wykonano na próbach pochodzących z doświadczenia polowego przeprowadzonego w latach 2002-2004 na glebie o składzie piasku gliniastego lekkiego. Eksperyment

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założono metodą losowanych podbloków w trzech powtórzeniach. Porównywano dwa sposoby uprawy roli – tradycyjną i uproszczoną oraz siedem sposobów odchwaszczania z udziałem herbicydów i ich mieszanin: Plateen 41,5 WG, Plateen 41,5 WG + Fusilade Forte 150 EC, Plateen 41,5 WG + Fusilade Forte 150 EC + adiuwant Atpolan 80 EC, Barox 460 SL, Barox 460 SL + Fusilade Forte 150 EC, Barox 460 SL + Fusilade Forte 150 EC + adiuwant Atpolan 80 EC, obiekt kontrolny pielęgnowany mechanicznie. Zawartość magnezu i jego pobranie z plonem bulw ziemniaka zależały istotnie od sposobów uprawy roli, sposobów odchwaszczania i warunków atmosferycznych w okresie wegetacji. Więcej magnezu zawierały bulwy ziemniaka zebrane z obiektów uprawy uproszczonej – średnio 1,771 g kg^{-1} w porównaniu z tradycyjną – średnio 1,762 g kg^{-1}. Herbicydy i ich mieszaniny zastosowane do odchwaszczania plantacji ziemniaka podwyższały istotnie zawartość magnezu w bulwach – średnio o 2,7% i jego pobranie z plonem bulw – średnio o 11,0% w porównaniu z obiektem kontrolnym pielęgnowanym mechanicznie.

Słowa kluczowe: bulwy ziemniaka, magnez, zawartość, pobranie.

INTRODUCTION

Potato tubers contain 1-1.2% mineral compounds, mainly potassium, phosphorus, magnesium and calcium. Consumption of 200g of potatoes satisfies 12-30% of human needs with respect to these elements (Leszczyński 2000). Mineral element content in potato tubers is affected by cultivar properties (Karim et al. 1997, Prośba-białczyk et al. 2002, Zarzecka et al. 2002), agronomic factors (Rogozińska et al. 1995, Wyszkowski 1996, Klikocka 2001, Nowak et al. 2004) and weather conditions over the growing period (Czekała, Gładysiak 1995, Zarzecka, Gąsiorowska 2002). The results of studies concerning impact of herbicides and soil cultivation methods on macroelement content in tubers vary as much as the researchers' opinions (Ceglarek, Księżak 1992, Klikocka 2001). Thus, the objective of the present work has been to determine the effect of soil tillage methods and weed control methods in potato cultivation on magnesium content and its removal by potato tuber crop.

MATERIAL AND METHODS

The experimental material consisted of cultivar Wiking edible potato tubers obtained from a field experiment conducted in 2002-2004. The experiment was established on light loamy soil and belonged to the very good rye complex. Selected chemical properties (organic matter, pH, available forms of phosphorus, potassium and magnesium) of the experimental field soil are presented in Table 1. The experiment was designed as randomised sub-blocks with three replications. The following factors were examined: I – two methods of soil tillage: conventional and simplified, II – seven methods of weed control including herbicide application (Table 2). Uniform mineral and or-

Table 1

Chemical and physical characteristics of the soil

Specification	2002	2003	2004
Organic matter (g kg^{-1})	11.3	11.3	11.5
pH 1 mol KCl dm^3	6.5	6.4	5.6
Content P (mg kg^{-1})	38.8	43.0	62.5
Content K (mg kg^{-1})	150.3	102.2	103.9
Content Mg (mg kg^{-1})	70.0	157.0	159.0

Table 2

Experimental factors

I. Tillage systems	1. Traditional (skimming + fall ploughing + harrowing + + cultivating + harrowing) 2. Simplified (skimming + cultivating)
II. Weed control methods	1. Control object - mechanical weeding until and after potato rising 2. Plateen 41.5 WG (metribuzin + flufenacet) 2.0 kg ha^{-1} 3. Plateen 41,5 WG (metribuzin +flufenacet) 2.0 kg ha^{-1} + + Fusilade Forte 150 EC (fluazyfop-P-butyl) 2.5 dm^3 ha^{-1} (mixture) 4. Plateen 41,5 WG (metribuzin +flufenacet) 1.6 kg ha^{-1} + + Fusilade Forte 150 EC (fluazyfop-P-butyl) 2.0 dm^3 ha^{-1} + + adjuvant Atpolan 80 EC 1.5 dm^3 ha^{-1} (mixture) 5. Barox 460 SL (bentazone + MCPA) 3.0 dm^3 ha^{-1} 6. Barox 460 SL (bentazone + MCPA) 3.0 dm^3 ha^{-1} + Fusilade Forte 150 EC (fluazyfop-P-butyl) 2.5 dm^3 ha^{-1} (mixture) 7. Barox 460 SL (bentazone + MCPA) 2.4 dm^3 ha^{-1} + Fusilade Forte 150 EC (fluazyfop-P-butyl) 2.0 dm^3 ha^{-1} + adjuvant Atpolan 80 EC 1.5 dm3 ha-1 (mixture)

ganic fertilization comprising 90 kg N, 32.9 kg P and 112.1 kg K ha^{-1}, and 25.0 t ha^{-1} (farmyard manure) was applied.

Magnesium content was determined in tuber dry matter following wet mineralization by the method of atomic absorption spectrophotometry (AAS). Magnesium removal by tuber yield was calculated from the product of tuber dry matter yield and Mg concentration. The results were statistically analysed with the analysis of variance and the significance of differences was determined using Tukey's test.

Weather conditions over the period of studies varied. The year 2002 was warm and wet with high temperatures during the months of tuber formation (July and August). The next year was also warm but the precipitation was insufficient as it reached barely 48.3% of long-term average sum. The year 2004 was wet and cold. Air temperatures and precipitation constituted 116.6 and 95.0%, respectively, of the long-term averages (Table 3).

Table 3

Weather conditions in 2002-2004

Years	Months						Mean value
	Apr	May	June	July	Aug	Oct	
Temperature (°C)							
2002	9.0	17.0	17.2	21.0	20.2	12.9	16.2
2003	7.1	15.6	18.4	20.0	18.5	13.5	15.5
2004	8.0	11.6	15.4	17.5	18.9	13.0	14.1
Multiyear mean 1981-1995	7.7	10.0	16.1	19.3	18.0	13.0	14.0
Rainfall (mm)							Sum
2002	12.9	51.3	61.1	99.6	66.5	18.7	310.1
2003	13.6	37.2	26.6	26.1	4.7	24.3	132.5
2004	35.9	97.0	52.8	49.0	66.7	19.5	320.9
Multiyear mean 1981-1995	52.3	50.0	68.2	45.7	66.8	60.7	343.7

RESULTS AND DISCUSSION

Under conditions of the experiment the average magnesium content in potato tubers ranged from 1.645 to 1.940 g kg^{-1} (Table 4). Significant effect of the soil tillage methods, weed control methods and moisture and thermal conditions on the magnesium concentration occurred. Magnesium content in tubers was similar to the levels reported by other authors (Prośba-Białczyk et al. 2002, Kołodziejczyk, Szmigiel 2005, Tekalign, Hammes 2005, Kozera et al. 2006). In the present study the reduced soil tillage increased the accumulation of magnesium compared with the conventional tillage. In contrast, Klikocka (2002) recorded an increased magnesium content in tubers harvested from plots which had been conventionally cultivated in comparison with the simplified cultivation. In their studies Klikocka and Komisarczuk (2000) observed that the use of no-plough tillage increased magnesium content in spring triticale grain compared with the plough-based cultivation.

The herbicides applied to control weeds in potato fields increased magnesium concentration in tubers by an average of 2.7% compared with mechanical weed control. The highest content of the macroelement appeared in tubers of potato plants sprayed with mixtures of Plateen 41.5 WG + Fusilade Forte 150 EC (on average 1.798 g kg^{-1}), and Barox 460 SL + Fusilade Forte 150 EC (on average 1.788 g kg^{-1}). Similar changes were observed by Zarzecka, Gąsiorowska (2002), who used herbicide mixtures and two applications of weed control chemicals. In contrast, Klikocka (2002) found no influ-

Table 4

Content of magnesium in potato tubers (g kg^{-1} d.m.)

Weed control methods	Tillage systems		Years			Mean
	traditio-nal	simpli-fied	2002	2003	2004	
1. Control object	1.719	1.734	1.645	1.865	1.670	1.727
2. Plateen 41,5 WG	1.763	1.777	1.694	1.918	1.699	1.770
3. Plateen 41,5 WG + Fusilade Forte 150 EC	1.794	1.807	1.780	1.930	1.692	1.798
4. Plateen 41,5 WG + Fusilade Forte 150 EC + Atpolan 80 EC	1.738	1.743	1.677	1.890	1.657	1.741
5. Barox 460 SL	1.765	1.787	1.710	1.916	1.702	1.776
6. Barox 460 SL + Fusilade Forte 150 EC	1.792	1.785	1.720	1.940	1.705	1.788
7. Barox 460 SL + Fusilade Forte 150 EC + Atpolan 80 EC	1.769	1.769	1.694	1.895	1.714	1.769
Mean	1.762	1.771	1.703	1.909	1.691	1.767
Mean for 2-7 object	1.769	1.778	1.713	1.915	1.695	1.774
$LSD_{0.05}$ – between:						
tillage systems (I)						0.004
weed control methods (II)						0.021
years (III)						0.007
interaction (I xII)						0.008
(II x III)						0.038
(I x II x III)						0.035

ence of chemical or mechanical and chemical weed control on the level of magnesium in potato tubers. According to ROLA, KIELOCH (2001), pesticides sprayed in a field to protect crop plants do not usually differentiate significantly the content of macroelements in plants. However, when crops compete with weeds, they can more easily take up individual elements.

Our analysis of the effect of the weather conditions during the experiment revealed that the highest amount of magnesium was in the tubers harvested in the warm and dry 2003. Differentiation of the magnesium content in tubers depending on weather conditions has also been reported in the papers by KLIKOCKA (2001), ZARZECKA et al. (2002) and KOŁODZIEJCZYK, SZMIGIEL (2005).

Magnesium removal by potato tuber crop was influenced by the experimental factors and weather conditions over the study years (Table 5). More magnesium was removed from conventionally tilled plots (on average 14.78 kg ha^{-1}) compared with the plots where some cultivation operations were abandoned (on average 13.92 kg ha^{-1}). It resulted from the fact that higher potato tuber yield was harvested from conventionally-tilled plots. Increased removal of magnesium, compared with the mechanically-tilled control, also

Table 5

Uptake of magnesium with the yield of potato tubers (kg ha^{-1})

Weed control methods	Tillage systems		Years			Mean
	traditio-nal	simpli-fied	2002	2003	2004	
1. Control object	13.49	12.75	16.12	11.61	11.64	13.12
2. Plateen 41,5 WG	14.19	13.72	17.13	12.53	12.21	13.96
3. Plateen 41,5 WG + Fusilade Forte 150 EC	15.05	14.74	18.87	13.03	12.80	14.90
4. Plateen 41,5 WG + Fusilade Forte 150 EC + Atpolan 80 EC	15.95	14.66	18.76	12.98	14.17	15.30
5. Barox 460 SL	14.11	13.60	16.43	13.45	12.19	14.02
6. Barox 460 SL + Fusilade Forte 150 EC	14.94	13.74	16.40	13.78	12.85	14.34
7. Barox 460 SL + Fusilade Forte 150 EC + Atpolan 80 EC	15.43	14.20	16.97	14.36	13.12	14.82
Mean	14.78	13.92	17.24	13.11	12.71	14.35
Mean for 2-7 object	14.95	14.11	17.43	13.36	12.89	14.56
$LSD_{0.05}$ – between:						
tillage systems (I)						0.16
weed control methods (II)						0.74
years (III)						0.25
interaction (I xII)						0.29
(II x III)						1.29
(I x II x III)						n.s.

n.s. – not significant

occurred after herbicide application. The increase ranged from 0.84 kg ha^{-1}, following spraying with Plateen 41.5 WG (treatment 2) to 2.18 kg ha^{-1} following an application of a mixture of Plateen 41.5 WG + Fusilade Forte 150 EC + adjuvant Atpolan 80 EC, compared with the mechanical control. Higher magnesium uptake was mainly associated with higher potato yields in the plots where weeds were chemically controlled. Magnesium removal associated with tuber yield was similar to the values cited by GRZEŚKIEWICZ and MAZURCZYK (2001), ranging from 13.1 to 20.1 kg ha^{-1}.

CONCLUSIONS

1. Reductions in soil tillage increased magnesium content in potato tubers compared with the conventional tillage. However, the removal of the element by potato tuber crop was higher in conventionally tilled plots.

2. Herbicides applied in potato cultivation increased magnesium content in tubers compared with the control.

3. Magnesium concentration and its removal by potato crop increased as a result of intensification of weed control in potato field.

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EFFECT OF MAGNESIUM ON BENEFICIAL ORGANISMS

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Abstract

The paper presents the results of research on magnesium effect on beneficial enthomopathogenic fungi and nematodes as well as some predatory arthropods.

Magnesium fertilization of soil contaminated with heavy metals does not significantly affect numbers of the majority of epigeal invertebrates, but it may influence numbers of single species, favouring the occurrence of some (*Bembidion* sp.) while restricting the presence of others (*Harpalus rufipes* De Geer).

Magnesium synergism with heavy metal ions has been found to increase infectiveness and pathogenicity of enthomopathogenic fungi. Magnesium, in a dose of 160 $mg \cdot dm^{-3}$ present in the medium, significantly enhances pathogenicity of fungi, such as *Beauveria bassiana*, *Paecilomyces farinosus*, *Paecilomyces fumoso-roseus or Metarhizium ansopliae*. Increased pathogenicity of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* towards test insects has also been observed when magnesium ions were added to a solution in which these nematodes were kept. An effective magnesium dose differed depending whether the nematodes were used separately for test insects (450 $mg \cdot dm^{-3}$) or jointly with enthomopathogenic fungi (320 $mg \cdot dm^{-3}$). This protective effect of magnesium ions on beneficial microorganisms has also been observed in soil contaminated with heavy metals. An addition of magnesium to a solution in which enthomopathogenic nematodes were kept (160 $mg \cdot dm^{-3}$) and to a medium on which fungi were cultured (320 $mg \cdot dm^{-3}$) increased pathogenic abilities of these organisms in contaminated soil to a very high degree (10- to 300- fold higher than the natural heavy metal content in soil).

Keywords: magnesium, epigeal fauna, enthomopathogenic fungi, enthomopathogenic nematodes.

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WPŁYW MAGNEZU NA WYBRANE ORGANIZMY POŻYTECZNE

Abstrakt

W pracy zestawiono wyniki badań nad wpływem magnezu na pożyteczne grzyby i nicienie owadobójcze oraz wybrane stawonogi drapieżne.

Nawożenie magnezowe gleby skażonej metalami ciężkimi nie wpływa istotnie na liczebność większości grup bezkręgowców naziemnych, może jednak wpływać na liczebność pojedynczych gatunków, sprzyjając występowaniu jednych (*Bembidion* sp.), a ograniczając występowanie innych (*Harpalus rufipes* De Geer).

Stwierdzono synergizm magnezu z jonami metali ciężkich w podnoszeniu infekcyjności i patogeniczności grzybów owadobójczych. Magnez obecny w pożywce, w dawce 160 g·dm^{-3}, istotnie podwyższał patogeniczność takich grzybów, jak: *Beauveria bassiana, Paecilomyces farinosus, Paecilomyces fumoso-roseus, Metarhizium ansopliae*. Zaobserwowano również zwiększenie patogeniczności nicieni owadobójczych *Steinernema carpocapsae* i *Heterorhabditis bacteriophora* wobec owadów testowych po dodaniu jonów magnezu do roztworu, w którym przechowywaneo te nicienie. Efektywna dawka magnezu była zróżnicowana, zależnie od tego, czy nicienie stosowano samodzielnie wobec owadów testowych (450 mg·dm^{-3}), czy też wraz z grzybami owadobójczymi (320 mg·dm^{-3}).

Obserwowano także protekcyjny wpływ jonów magnezu na pożyteczne mikroorganizmy w warunkach gleby zanieczyszczonej metalami ciężkimi. Dodanie magnezu do roztworu, w którym przechowywano nicienie owadobójcze (160 mg-·dm^{-3}), i do pożywki, na której hodowano grzyby (320 mg·dm^{-3}), zwiększało zdolności patogeniczne tych organizmów w warunkach gleby skażonej metalami ciężkimi nawet w bardzo wysokim stopniu (10-300-krotnie wyższa zawartość metali ciężkich w glebie ponad zwartość naturalną).

Słowa kluczowe: magnez, fauna naziemna, grzyby entomopatogenicze, nicienie entomopatogeniczne.

INTRODUCTION

Both representatives of beneficial epigeal enthomofauna (*Carabidae, Staphylinidae, Arachnida*) and beneficial microorganisms (enthomopathogenic fungi and nematodes, such as: *Beauveria bassiana, Paecilomyces farinosus, Paecilomyces fumoso-roseus, Metarhizium ansopliae, Steinernema carpocapsae* and *Heterorhabditis bacteriophora*) are an important factor allowing to maintain populations of many harmful insects at a level which does not pose any threat to agronomic crops. The efficiency of these organisms may be modified by the effect of soil anthropogenic pollution such as heavy metals (JAWORSKA et al. 1996, JAWORSKA et al. 1997 a, c). Frequently, besides liming, magnesium fertilization of soils polluted with heavy metals is mentioned as a means to diminish their toxicity. Magnesium may be used for simple chemical stimulation of the pathogenicity of beneficial microorganisms.

This paper presents a compilation of the results of research on the effect of magnesium fertilization of soil contaminated with heavy metals on beneficial epigeal fauna and investigations on potential practical enhancement of the effectiveness of enthomopathogenic fungi and nematodes by means of their conditioning with magnesium.

MATERIALS AND METHODS

The paper comprises results of field and laboratory analyses. The field experiment was conducted in 2000 on an arable field in Zagaje Stradowskie, a village in the Świętokrzyskie Province. Broad beans (*Vicia faba* L. ssp. *maior)*, cv. White Windsor was cultivated on the following objects:

1. Control (soil with natural content of heavy metals: 8.2 mg Cu, 52.9 mg Zn, 28.2 mg Ni and 0.6 mg Cd$\cdot$kg^{-1}d.m. soil);

2. Soil contaminated with heavy metals dosed: 2 mg Cd, 15 mg Ni, 30 mg Cu, 70 mg Zn and 80 mg Pb$\cdot$kg^{-1} d.m. soil);

3. Soil receiving magnesium fertilizer (50 kg MgO$\cdot$ha^{-1}) and contaminated with heavy metals;

4. Soil receiving magnesium fertilizer (50 kg MgO$\cdot$ha^{-1}) and liming (2900 kg CaO$\cdot$ha^{-1}), contaminated with heavy metals.

Analyses on enthomopathogenic fungi and nematodes were conducted under laboratory conditions using various concentrations of magnesium in media for fungi culturing or in aqueous solutions for keeping nematodes. The methods were presented in detail in other papers (JAWORSKA, GOSPODAREK 2003 a, b, JAWORSKA et al. 1996, JAWORSKA et al. 1999).

RESULTS AND DISCUSSION

Soil contamination with heavy metals causes a decrease in the abundance of captured epigeal fauna (Figure 1). Magnesium fertilization and liming of contaminated soil does not significantly influence the counts of most analyzed invertebrate groups, but it may affect the numbers of individual species. In the case of small *Bembidion* (*Col., Carabidae*) beetles a slight increase in the number of trapped insects may be observed in soil contaminated with heavy metals after application of magnesium fertilization. On the other hand, a common *Carabidae Harpalus rufipes* De Geer species, which occurred as numerously in soil contaminated with heavy metals as in the control, was considerably less often trapped on the magnesium fertilized object. The data on the effect of liming on epigeal fauna are quite divergent. In some research, the number of trapped epigeal fauna after liming of the polluted soil increased. Differences in the response to various degrees of farming intensity were also noted among various *Carabidae* species (JAWORSKA 1997, PAŁOSZ 1998). On fields under the most intensive farming system, fewer *Carabidae* were trapped. The species which most strongly decreased in number under more intensive insecticidetreatments included *Carabus auratus* L., *Pterostichus cupreus* L., *Pterostichus melanarius* III. and *Amara* sp. On the other hand, *Pterostichus lepidus* Leske species was more

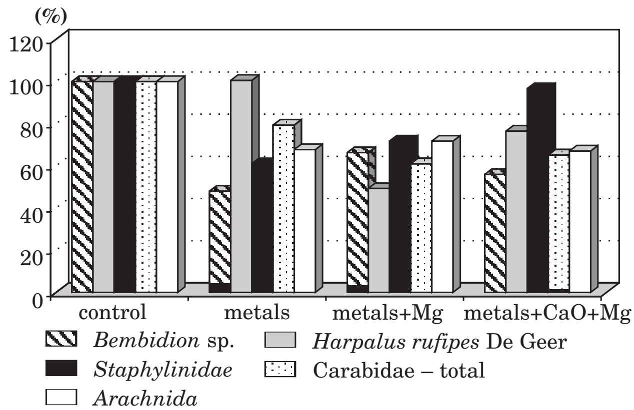

Fig. 1. Representatives of beneficial epigeal fauna in soil with natural heavy metal content and in metal contaminated soil receiving magnesium treatment (% in relation to the control)

numerously caught on a field where more intensive chemical protection was used. The latter fact was explained by the authors as a result of diversified phenology of this species occurrence and therefore its smaller exposure to pesticide poisoning. In the above research both *Ophonus rufipes* De Geer species and *Bembidion* sp. genus were significantly less numerously trapped on the most intensively farmed fields in comparison with extensive tillage, although a greater (3-fold) decline was found for *Bembidion* sp. beetles.

Enthomopathogenic fungi are quite tolerant to heavy metal presence in the environment (Roux et al.1993). The limits of this tolerance may change under the influence of interaction between metal ions (Jaworska, Tomasik 1999). Investigations on the effect of various metals on the growth of enthomopathogenic *Paecilomyces fumoso-roseus* fungus colonies and its infectiveness towards *Bruchus pisorum* L. evidenced a marked (*ca* 25%) increase in infectiveness of this fungus owing to 160 mg·dm^{-3} supplement of Mg ions added to the medium (Table 1). The effect occurred despite the fact that magnesium did not reveal positive influence on the growth of fungal colonies. The result of combining Mg with other metals on the fungus was also investigated. Various metal ions previously added to the medium caused an inhibition of fungal colony growth and, in most cases, a decline in its infectiveness. On the other hand, a supplement of magnesium ions added to media which had already contained single metals (Al, Fe, V, Cr or Pb) alleviated the toxic effect and enhanced the infectiveness of fungi as compared with the control.

The research on potential chemical stimulation of enthomopathogenic activity of other fungal species towards a legume pest *Sitona lineatus* L. demonstrated that Mg ion in the medium (160 mg·dm^{-3}) significantly raised pathogenicity of such fungi as *Beauveria bassiana, Paecilomyces farinosus, Paecilomyces fumoso-roseus* or *Metarhizium ansopliae* (Table 1).

Table 1

Effect of mangesium on enthomopathogenic fungi and nematodes

Investigated feature	Control	Mg
Mortality (%) of *Bruchus pisorum* L. on the 5th day after its contact with P. *fumosoroseus* grown on agar broth with added Mg ions (160 mg·dm^{-3})	73.30 *b**	93.30 *a*
Mortality (%) of *Sitona lineatus* L. on the 5th day after its contact with enthomopathogenic fungi grown on a media with Mg ions (160 m·dm^{-3}):		
Beauveria bassiana	46.60 *a*	100.0 *b*
Paecilomyces farinosus	36.60 *a*	66.70 *b*
Paecilomyces fumoso-roseus	36.60 *a*	50.00 *a*
Metarhizium ansopliae	43.30 *a*	63.30 *b*
Mortality (%) of *Galleria mellonella* on the 5th day after its contact with enthomopathogenic fungi and nematodes grown on a medium with Mg ions (mg·dm^{-3}):		
Beauveria bassiana	24.20 *a*	
Mg 450		38.70 *a*
Mg 320		71.30 a
Mg 160		62.10 *a*
Steinernema carpocapsae	65.10 *a*	
Mg 450		95.10 *b*
Mg 320		76.4 0 *ab*
Mg 160		81.00 *ab*
S. carpocapsae+B. bassiana	70.20 *ab*	
Mg 450		87.60 *ab*
Mg 320		99.00 *b*
Mg 160		78.80 *a*
Effect of conditioning nematodes in aqueous solutions of Mg (160 mg·dm^{-3}) ions on their pathogenicity (mortality (%) of *Sitona lineatus* L. on the 7th day after its contact with enthomopathogenic nematodes)		
Steinernema carpocapsae	75.10 *a*	100.0 *b*
Heterorhabditis bacteriophora	47.40 *a*	91.70 *b*
Effect of conditioning nematodes in aqueous solutions of Mg (160 mg·dm^{-3}) ions on their reproduction inside the S. *lineatus body* (number of nematode invasive larvae per mg body weigth)		
Steinernema carpocapsae	77.00 *a*	144.0 *b*
Heterorhabditis bacteriophora	111.0 *a*	184.0 *b*
Mortality (%) of *Galleria mellonella* on the 2nd day after its contact with enthomopathogenic nematodes (conditioned in aqueous solutions of Mg (mg·dm^{-3}):		
Steinernema carpocapsae	80 *a*	
32		100 *b*
320		100 *b*
Heterorhabditis bacteriophora	80 *a*	
32		100 *b*
320		100 *b*

*Means for each property marked with the same letter do not differ at $P > 0.05$.

Heavy metals, such as cadmium, copper, zinc and lead, cause increased mortality of enthomopathogenic nematode infective juveniles as well as decrease their pathogenicity towards test insects and reproduction (JAWORSKA, GORCZYCA 2002). In an experiment using soil strongly contaminated with heavy metals, decreased infectiveness of these beneficial microorganisms was found (ROPEK, GORCZYCA 2000). Analogously to enthomopathogenic fungi, a beneficial effect of magnesium ions was found for nematodes, visible as their increased pathogenicity (JAWORSKA et al. 1996, JAWORSKA et al. 1997 a,b). In experiments on stimulating enthomopathogenic activity of fungi and nematodes applied jointly on *Galeria mellonella* test insects it was found that, among three tested Mg ion concentrations, the concentration of 320 mg$\cdot$dm^{-3} increased most infectiveness of the analyzed microorganisms. When only *Steinernema carpocapsae* nematodes were used for the test insects, the highest mortality of *Galeria mellonella* caterpillars was obtained at the highest Mg ion concentration (450 mg$\cdot$dm^{-3}) used for the nematode stimulation (Table 1). An advantageous effect of keeping *S. carpocapsae* and *Heterorhabditis bacteriophora* nematode in aqueous Mg solution (160 mg$\cdot$dm^{-3}) was also found when various development stages of *Sitona lineatus* L. were controlled using these organisms (Table 1). In this case, magnesium acted positively by increasing reproduction of the nematode species.

The experiments on magnesium interaction with heavy metal ions added to soil towards enthomopathogenic fungi and nematodes revealed that magnesium supplement to a solution used for keeping nematodes (160 mg$\cdot$dm^{-3}) and to a medium for fungi (320 mg$\cdot$dm^{-3}) enhanced pathogenicity of both organisms in soils with heavy metals 10- to 300-fold exceeding their natural content (JAWORSKA 1999).

CONCLUSIONS

1. Magnesium fertilization of soil contaminated with heavy metals does not affect significantly the number of most epigeal invertebrate groups, although it may influence single species, favouring some (*Bembidion* sp.) but limiting the occurrence of others (*Harpalus rufipes* De Geer).

2. A supplement of magnesium ions added to a solution used for keeping enthomopathogenic nematodes or to a medium for fungal cultures may be recommended as a simple method to stimulate biological activity of these microorganisms.

3. Magnesium can also protect the above microorganisms in soil polluted with heavy metals.

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THE EFFECT OF PROGRESSIVE ACIDIFICATION OF LESSIVE SOIL ON ZINC CONTENT AND ITS TRANSLOCATION IN SOIL PROFILE

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Abstract

In Poland 11% of soils feature elevated zinc content or slight zinc contamination. This investigation aimed at estimation of the effect of progressive acidification of slightly zinc-contaminated soils on zinc content in plants and translocation of this metal downwards the soil profile. The study involved a two-year lysimetric experiment on lessive soil. The amount of zinc indicating slight soil contamination was introduced into 0.2 m of topsoil, which was subjected to progressive acidification with sulfuric acid solution in the course of the experiment.

Zinc content proved to considerably increase in plants (barley straw and maize) only under strong acidification. Soil reaction did not significantly influence the zinc content in soil, both total and assayed in HCl zinc forms, while a considerable increase in easily soluble zinc forms (in $CaCl_2$ solution) occurred on strongly acidified soils.

Considering the whole research period, increasing soil acidification did not result in any alterations involving zinc content in Bbr and C horizons of soil profiles (below 30 cm).

Key words: Zn, soil reaction, plants, lysimeters.

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WPŁYW SYSTEMATYCZNEGO ZAKWASZANIA GLEBY PŁOWEJ NA ZAWARTOŚĆ CYNKU W ROŚLINACH ORAZ JEGO PRZEMIESZCZANIE W PROFILU GLEBOWYM

Abstrakt

W Polsce 11% gleb charakteryzuje się podwyższoną zawartością lub słabym zanieczyszczeniem cynkiem. Celem pracy było określenie wpływu systematycznego zakwaszania gleby słabo zanieczyszczonej cynkiem na zawartość tego pierwiastka w roślinach i jego przemieszczanie w głąb profilu glebowego. Badania prowadzono w trakcie dwuletniego doświadczenia lizymetrycznego na glebie płowej. Do warstwy gleby 0-20 cm dodano cynk w ilości odpowiadającej słabemu zanieczyszczeniu tym pierwiastkiem. W czasie badań glebę systematycznie zakwaszano, stosując roztwór kwasu siarkowego.

Stwierdzono, że zawartość cynku w roślinach (jęczmień-słoma i kukurydza) istotnie wzrosła tylko na najbardziej zakwaszanych obiektach. Odczyn gleby nie wpływał istotnie na zawartość całkowitych i oznaczanych w HCl form cynku w glebie. Natomiast zawartość łatwo rozpuszczalnych form cynku (w roztworze $CaCl_2$) istotnie wzrosła w najbardziej zakwaszonych glebach. Rosnące zakwaszenie gleby nie wpłynęło w trakcie badań na zawartość cynku w poziomach Bbr i C profilu glebowego (poniżej 30 cm).

Słowa kluczowe: Zn, formy, pH gleby, rośliny, lizymetry.

INTRODUCTION

In Poland, soils characterized by elevated zinc content (I°) or slightly contaminated with zinc (II°) constitute 11 % of agricultural acreage (MERCIK at al. 2003). Mobility of this metal is related to soil properties, especially its reaction (GORLACH, GAMBUŚ 1991, MERCIK et al. 2003). Therefore, prolonged acidification of soil potentially facilitates increased accumulation of zinc in plants and its translocation downwards the soil profile.

The aim of the work was to examine the effect of progressive soil acidification on zinc accumulation in plants, as well as on translocation of this metal from topsoil downwards the soil profile under slight soil contamination with zinc.

MATERIAL AND METHODS

In 2002-2003, a study was completed based on a lysimetric experiment. Lysimeters of 0.5 m^2 area and 1m depth were filled with lessive soil maintaining its natural genetic levels. Selected physicochemical soil properties are shown in Table 1. When filling lysimeters with soil, zinc in the form of $Zn(NO_3)_2$ was introduced to 0-20 cm topsoil in the amount indicating slight soil contamination (II°) with this metal. In this experiment it reached 350 mg $Zn \cdot kg^{-1}$. The experimental design involved 5 treatments (each with three

Table 1

Some properties of lessive soil used in experiment

Horizon, depth (cm)	Fraction (%)		C org. ($g \cdot kg^{-1}$)	pHKCl	CEC* ($mmol(+) \cdot kg^{-1}$)	Zn content ($mg \cdot kg^{-1}$)		
	<0.02	<0.002				soil extract		
	mm	mm				$HClO_4$	HCl	$CaCl_2$
Ap (0-30)	17	9	9.0	5.8	106	36.4	16.5	2.72
Bbr (30-60)	15	6	4.2	5.5	68.3	19.9	6.07	0.85
C (60-90)	10	4	1.5	5.4	43.5	7.15	2.42	0.60

* CEC – cation exchangeable capacity

replications), representing various effects produced by factors regulating soil reaction. According to the experimental design, the soil in treatment 1 received two limings with a mixture of calcium carbonate and magnesium, following 0.5 Hh (spring and fall 2002) in order to prevent its acidification. The soil in objects 3-5 was systematically acidified using increasingly higher quantities of sulfuric acid solution which, expressed as (S), amounted yearly to 50, 150 and 300 $kg \cdot ha^{-1}$, respectively. Diluted sulfuric acid was spread on soil surface. Thus, the topsoil reaction (0-20 cm) was diversified during the investigation, with the pH values ranging from 4.7 to 6.1 (Table 2). Plants subjected to the test were spring barley (2002) and winter rape (2002/2003). The latter, because of its unsatisfactory winter survival, was removed and then maize was sown on that stand to be harvested in the early dough stage. In the years of the experiment, monthly precipitation generally did not exceed average multi-year values, while at the turn of 2002 and 2003 and in the subsequent months winter – spring drought occurred. The period between February and July received merely 47% of the average precipitation for many years. When the experiment was completed, soil samples were collected from the whole profile at every 10 cm to undergo the following analysis: pH was assayed with the potentiometric method in 1 mole $KCl \cdot dm^{-3}$, total Zn content was determined by sample etching in perchloric acid (Anonim 1987) and soluble forms of this metal were assayed in 1 mole $HCl \cdot dm^{-3}$ (GEMBARZEWSKI et al. 1987) and 0.01 mole $CaCl_2 \cdot dm^{-3}$ (NOVOZAMSKY et al. 1993). Plant samples were subjected to mineralization according to the microwave technique and zinc was assayed through the AAS method. Statistical analysis was applied using AWAR computer program.

Table 2

Zinc content in soil ($mg \cdot kg^{-1}$) after completion of the experiment as dependent on soil pH and soil extract solution

Soil layer (cm)	Treatment					$LSD_{0.05}$
	1	2	3	4	5	
pH value						
0-20	6.1	5.6	5.4	5.0	4.7	-
20-30	5.9	5.8	5.7	5.3	4.9	-
30-40	5.6	5.6	5.6	5.5	5.5	-
$HClO_4$ (ANONIM 1976)						
0-20	185.2	184.6	180.5	182.7	179.3	n.s.
20-30	114.7	117.3	119.1	117.6	119.8	n.s
30-40	19.6	21.0	20.5	20.0	21.0	n.s
1 mol HCl (GEMBARZEWSKI et al. 1987)						
0-20	153.6	154.2	156.8	155.3	158.4	n.s
20-30	73.1	74.4	74.2	74.4	77.8	n.s
30-40	6.40	6.83	6.45	6.68	6.97	n.s
0.01 mol $CaCl_2$ (NOVOZAMSKY et al. 1993)						
0-20	19.11	20.95	21.34	26.58	34.96	2.87
20-30	11.07	11.34	11.39	12.92	17.18	1.65
30-40	0.88	0.88	0.90	0.90	0.95	n.s.

RESULTS AND DISCUSSION

Gradual acidification of soil in the conditions of its slight contamination with zinc did not significantly affect yielding of cultivated plants (Table 3). However, on the most acidified treatments (4 and 5) zinc content in barley straw and maize did considerably increase as compared to non-acidified ones (1 and 2). In spite of this, the critical values taken for assessment of plant for consumption or fodder were not exceeded (KABATA-PENDIAS et al. 1993).

Total zinc content in soil and that assayed in HCl extract were not significantly dependent on soil reaction (Table 2). However, opinions regarding the influence of soil reaction on zinc solubility are contrary. MERCIK et al. (2003) demonstrated no effect on the total Zn form and the one extracted with HCl solution, while GORLACH and GAMBUŚ (1991) and KIEKENS (1995) reported that regardless the form of zinc, its solubility depended on soil reaction and decreased proportionally to the increase in pH value.

Table 3

Zinc content in dry matter yields of barley and maize ($mg \cdot kg^{-1}$ DM) and yield data (g per lysimeter)

Treatment	Yield			Zn content		
	barley		maize	barley		maize
	grain	straw		grain	straw	
1. Ca-2 x 0.5 Hh	174.2	280.9	870.6	29.14	32.61	37.25
2. Ca-0; S-0	162.1	274.3	867.4	31.05	34.02	39.93
3. S-50 kg*	171.6	279.1	873.5	31.43	34.14	40.78
4. S-150 kg*	184.8	287.4	892.1	32.09	36.07	44.92
5. S-300 kg*	180.3	281.2	914.7	33.68	39.12	54.16
LSD0.05	n.s.	n.s.	n.s.	n.s.	3.21	4.15

*Dose of sulfuric acid solution expressed as sulfur – S ($kg \cdot ha^{-1} \cdot year^{-1}$)

The smallest amount of zinc was assayed in $CaCl_2$ extract (Table 2). On the treatments characterized by slightly acid soil reaction (1 and 2), such zinc equalled 10.8% and when the soil reaction was 4.7 pH it reached 19.5% of the total zinc value. Therefore, our results are in agreement with the literature data, which implicate that on acid soils the quantity of soluble zinc forms increase, which favours availability of this element to plants (Gorlach, Gambuś 1991, Mercik et al. 2003).

Under progressive soil acidification, values of zinc forms assayed in soil profile below 30 cm did not increase. This can suggest that when soil contamination with zinc is slight, zinc accumulates in the arable layer but is not translocated downwards soil profile does. However, the present results could have been affected by the weather conditions. It is apparent that translocation of components in soil is strongly related to water content in soil as well as predominant direction of its movement (Koc et al. 2003). Taking into account the amount and distribution of precipitation in the course of this investigation, it is possible to hypothesize that rinsing was limited, which can be confirmed by the absence of soil leachate in the years when this experiment was conducted.

CONCLUSIONS

1. Gradual soil acidification under slight soil contamination with zinc did not significantly influence the yielding of cultivated plants.

2. Zinc content in plants, mainly in barley straw and in maize, considerably increased only on highly acidified treatments, but its amounts did not

exceed the critical values established for plants for consumption (barley grain) or fodder (barley straw and maize).

3. Total zinc content and zinc assayed in HCl extract in soil were not dependent on soil reaction, while on the most acidified treatments, the zinc content assayed in $CaCl_2$ extract did significantly increase in Ap (0-30 cm) horizon.

4. Under progressive soil acidification, no evidence emerged suggesting increase in zinc forms assayed in Bbr and C horizons of soil profiles (below 30 cm).

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INFLUENCE OF NPK FERTILIZATION ENRICHED WITH S, Mg, AND MICRONUTRIENTS CONTAINED IN LIQUID FERTILIZER INSOL 7 ON POTATO TUBERS YIELD (*SOLANUM TUBEROSUM* L.) AND INFESTATION OF TUBERS WITH *STREPTOMYCES SCABIES* AND *RHIZOCTONIA SOLANI*

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Abstract

Elemental sulphur and Kieserite fertilization have been demonstrated to improve potato tuber (*Solanum tuberosum*) yield quality and resistance against *Streptomyces scabies*; the bacterial effect was attributed to reduced soil pH. So far, no information has been available about the influence of S, Mg and supply of micronutrients on bacterial and fungal diseases of potato plants. Field trials performed in a split-plot design with varied fertilization treatments (NPK with/without S and Mg and microelements B, Zn, Mn, Cu fertilization) including three potato cultivars were conducted in the south eastern region (near Zamość) of Poland in 2004-2006. The application of S and Mg and micronutrients decreased the tuber infection rate and severity of *Streptomyces scabies* and *Rhizoctonia solani*, while increasing potato tuber yield. Generally, tuber yield and wholesomeness were mostly related to a genotype (cultivar), mineral fertilization treatments and their interaction with a cultivar.

Key words: potato tuber, mineral fertilization treatments, *Streptomyces scabies*, *Rhizoctonia solani*.

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WPŁYW WZBOGACENIA NAWOŻENIA NPK DODATKIEM S, Mg I MIKROELEMENTÓW ZAWARTYCH W PŁYNNYM NAWOZIE INSOL 7 NA PLON I PORAŻENIE BULW ZIEMNIAKA (*SOLANUM TUBEROSUM* L.) PRZEZ *STREPTOMYCES SCABIES* I *RHIZOCTONIA SOLANI*

Abstrakt

Nawożenie ziemniaka siarką elementarną i kizerytem podwyższa plon bulw, poprawia ich jakość i odporność na porażenie przez *Streptomyces scabies*. Obniżenie odczynu gleby (pH) wskutek nawożenia S-elementarną może ograniczać wpływ chorób grzybowych. Jednakże brak jest szerszych informacji o bezpośrednim wpływie łącznego nawożenia S i Mg na wzrost odporności na porażenie bulw ziemniaka przez choroby bakteryjne i grzybowe. Eksperyment polowy na glebie brunatnej wyługowanej przeprowadzono w latach 2004-2006 w płd.-wsch. rejonie Polski (k. Zamościa). Pod 3 odmiany ziemniaka zastosowano następująco zróżnicowane nawożenie mineralne: NPK oraz NPK z dodatkiem S, Mg i mikroelementów: B, Zn, Mn, Cu. Wzbogacenie NPK w S i Mg oraz mikroelementy spowodowało zmniejszenie porażenia bulw przez *Streptomyces scabies* i *Rhizoctonia solani* oraz zwiększenie plonu bulw ziemniaka. Generalnie zdrowotność i plon bulw były najsilniej zależne od odmiany, następnie od nawożenia mineralnego oraz jego współdziałania z odmianami.

Słowa kluczowe: bulwy ziemniaka, poziom nawożenia mineralnego, *Streptomyces scabies*, *Rhizoctonia solani.*

INTRODUCTION

Mineral nutrients are routinely applied to boost crop yields and improve overall plant health and quality. The nutrition of plants largely determines their resistance or susceptibility to disease, histological or morphological structure and properties, and the virulence or ability of pathogens to survive. Mineral nutrients are frequently the first and foremost line of defence against plant diseases and influence all parts of the disease "pyramid" (HUBER, HANEKLAUS 2007, DATNOF et al. 2007). Plants have developed different forms of resistance. Nutrient-induced resistance was observed for phosphate, silicone and sulphur (KLIKOCKA et al. 2005, SALAC 2005). The mineral plant nutrition can potentially improve the stress resistance of plants. The mechanisms for the sulphur-induced resistance (SIR) of plants against fungal diseases include the increased synthesis of natural components, degradation of glycosides, synthesis of new components and the hypersensitive response (HANEKLAUS et al. 2002). LAMBERT et al. (2005) report that nutrient balance can decrease the severity of many important potato diseases and that certain practices, such as maintaining low pH for scab control, have been followed for this purpose.

Sulphur and magnesium fertilization increased yield of potato tubers, improved tuber quality and resistance against *Streptomyces scabies*, whereby the bactericidal effect was attributed to a reduced soil pH by elemental S applications (GRZEBISZ, HÄRDTER 2006, LAMBERT et al. 2005, PAVLISTA 1995, PRA-

KASH et al. 1997, ROGOZIŃSKA 1991). SAWICKA and KROCHMAL-MARCZAK (2008) studied influence of liquid fertilizer Insol 7 on diseases of potato tubers. So far, however, no information has been available about the influence of S and Mg supply on bactericidal and fungal diseases in potato. The aim of the present research has been to quantify the influence of fertilization treatments (with and without S and Mg and fertilization with micronutrients: B, Zn, Mn, Cu) on the tuber yield and infestation of three potato tuber cultivars with *Streptomyces scabies* and *Rhizoctonia solani* as a contribution of plant nutrition strategies for healthier plants.

The pathogens Streptomyces scabies and Rhizoctonia solani

The skin disease „common scab" is caused by the bacterium *Streptomyces scabies*. Virulent species of these otherwise saprophytic filamentous bacteria produce a toxin, thaxtomin, which kills patches of periderm cells and elicits production of newly suberized layers, which leave raised or pitted scabs on the tuber surface (BŁASZCZAK et al. 2005, FAUCHER et al. 1992, LAMBERT et al. 2005). The disease occurs in all regions of potato cultivation, particularly on light, friable, dry and alkaline soils (BRAZDA 1995, ELPHINSTONE 2007). In Poland, as well as in other countries, up to 70% of tubers may be infested by potato scab subject to the cultivation region and variety (GUGAŁA et al. 2007, KEINATH, LORIA 1989, KLIKOCKA et al. 2005, PUŁA, ŁABZA 2007, SALAZAR 2006). The optimum conditions for common scab development are air temperatures of 23-25°C. An Insufficient nutrient supply (Mn, K, Mg, Zn, B) favours infestation with *Streptomyces scabies* (BRAZDA 1995). LAMBERT et al. (2005) suggested a positive relationship between higher periderm Ca content and scab, despite the general benefits of increasing calcium availability in alkaline soils. A reduction of soil pH by gypsum and elemental S or ammonium sulphate applications reduced infestation with *Streptomyces scabies* (KLIKOCKA et al. 2005, LAMBERT et al., 2005, PAVLISTA 1995). BARNES (1972) applied 0.5 MT ha^{-1} elemental sulphur and reduced scab by 27% with a pH change of 6.1 to 5.4. In a subsequent trail, 0.5 or 1 MT ha^{-1} elemental S reduced common scab by 50% with the pH reductions from 5.2 to 4.6 and 4.2. The author, however, cautioned against over-application of elemental S. Reduction of elemental sulphur to hydrogen sulphide, which is toxic to *S. scabies in vitro*, has been considered as a possible mechanism of S-induced scab reduction. This conversion requires anaerobic conditions, such as might result from waterlogging, and its practical significance under typical field conditions is unknown (LAMBERT et al. 2005). DAVIS et al. (1976a) report that growing potatoes without any P fertilizer substantially increased scab compared to the other four P treatments, which ranged from 84 to 336 kg ha^{-1} P_2O_5. Scab could not be reduced by increasing or decreasing the standard rate of phosphorus. In another study, various rates of elemental sulphur decreased scab when irrigation thresholds were 100 kPa or above. Elemental S increased yield by an average of 20% and sulphur treatment also increased petitole P (DAVIS et al. 1976b). Other elements have been investigated for control of common scab, particularly manganese (KEINATH, LORIA 1989), whose availability in soil increases with acidity and soil moisture, supporting the hypothesis that Mn toxicity to *Strpetomyces scabies* is a major mechanism in the pH and irrigation effects and that Mn applications would further reduce scab. LAMBERT et. al. (2005) concluded that Mn improves host resistance to tuber pathogens, possibly in its role as a cofactor with enzymes involved in oxidation/reduction or with direct oxidation of phenolics. In this case, plant manganese concentration adequate for yield may be suboptimal for disease resistance.

In compliance with the standards and norms for the international trade within the European Union, which Poland observes, the area of the tuber surface affected by *Streptomyces*. Gus may not exceed 1/5 of total tuber surface. For potato seeds, infection by scab cannot exceed 5% of the total surface (ZGÓRSKA, FRYDECKA-MAZURCZYK 2002).

Rhizoctoniose or „stem cancer" caused by the fungus *Rhizoctonia solani* is known as one of the most widespread diseases of cultivated plants, particularly potatoes, root vegetables, cabbage, tomatoes, cereals and grasses. *R. solani* is a soil borne pathogen. The symptoms characteristic for potato infestation include rotting sprouts, stem dry rotting and smallpox (READ et al. 1889, YAO 2002). The fungus *R. solani* is tuber coloniser, most easily identified by small black sclerotia (black scurf stage) on the tuber surface. BŁASZCZAK et al. (2005) used scanning electron microscopy to investigate different types of necroses in potato tubers infected by rhizoctoniose. Cultivar Irga showed superficial necrotic symptoms caused by *Rhizoctonia* ssp. Fungal necrosis caused strong disintegration of cell walls with cell lysis. Additionally, tubers are small in size, often deformed and smallpoxed (READ et al. 2005).

Usually, about 14-20% of the tubers show symptoms of the infestation with rhizoctoniose with variations dependent on the cultivation region and variety (KURZINGER 1995, SALAZAR 2006). In Poland, however, up to 40% of the tuber surface may be affected by infestation with this pathogen (KLIKOCKA 2001, PUŁA, ŁABZA 2007).

Rhizoctonia solani survives in the soil during winter in form of sclerotia or mycelia on residues of infested plants and on potato seedlings, respectively. The optimum temperature for plant infestation is 15-18^{o}C (BŁASZCZAK et al. 1997). Under disadvantageous growing conditions, such as high moisture and low temperature in the soil combined with poor plant growth, the disease spreads soon after planting potatoes. Early planting of potatoes increases the risk of rhizoctoniose. For planting, only those tubers should be selected which are covered with no more than 20% sclerotia on the surface (HÄNI et al. 1976). Common agronomic measures to reduce the infestation risk include the choice of suitable forecrops, careful application of herbicides (RADTKE 1994, PUŁA, ŁABZA 2007) and optimum planting depth (STACHEWICZ 1996). JABŁOŃSKI (2006) observed that rhizoctoniose of tubers increased after simplification of soil cultivation. Although the disease may cause important losses, little work has been done on nutritional effects. Manganese at 62 kg ha^{-1} reduced from 25% to 11%. the incidence of *Rhizoctonia solani* black scurf on tubers No differences in number of stem lesions were obtained over a range of 0 to 250 kg N ha^{-1} (LAMBERT et al. 2005), but RĘBARZ and BORÓWCZAK (2007) reports that increase of nitrogen doses from 0 to 180 kg N ha^{-1} cased the decrease of occurrence of black scurf. KLIKOCKA et al. (2005) observed that sulfur application reduced the fungal pathogen of potato tubers. Other observations also emphasize the preventive role of sulfur against the fungal diseases (DATNOFF et al. 2007, HUBER, HANEKLAUS 2007, SALAC 2005, HANEKLAUS et al. 2002). The agronomic measures, beside the chemical methods and cultivation of resistant varieties, are of the utmost importance in prevention of plant diseases. It has been shown that mineral fertilization (with macro- and micronutrients) promotes natural plant resistance mechanisms, similarly to application of silicon, aluminum and sulfur (DATNOFF et al. 2007, HUBER, HANEKLAUS 2007).

MATERIAL AND METHODS

The description of experiment sites

The field experiments on potatoes were conducted at Malice (N 50°42'; E 23°15'), a village near Zamość in Poland, in the years 2004-2006. The experiment was carried out on leached brown soil with loamy silty soil texture. The soil characteristics (according to SILANPÄÄ 1982) were 13% clay, 29% silt, 58% sand, 8.4 g kg^{-1} of total-C content (by dry combustion; LECO EC-12®, model 752-100) and pH 5.3 (potentiometrically in 0.01 M $CaCl_2$ suspension using a Methrohm 605 pH-meter). Total-N was on average 0.8 g kg^{-1} (by Kjeldahl extraction method), plant available P 72.6 mg kg^{-1} and K 63.1 mg kg^{-1} (vanadium-molybdene by Egner-Riehm method), phosphorus (by the colorometric method) and potassium (by photometry), plant available Mg 37.0 mg kg^{-1} (by Schachtschabel method), plant available S-SO_4 – 11.5 mg kg^{-1} (extracted by 0.025 m KCl and determined in an ion chromatograph) (BLOEM 1998).

Throughout the last two decades, the air temperature sum and the total precipitation in the growing seasons averaged 2344°C and 386 mm. The total rainfall in the seasons of 2005 and 2006 was lover than the long-term sum. In 2004, the total rainfall was similar to the long-term sum. The monthly means of the air temperatures in the growing seasons in 2004-2006 were much higher than over the long term. June, July and August were particularly hot months. Generally, the growing season in 2004 was very warm and wet, while the vegetation seasons of 2005-2006 were very hot and dry (Table 1).

Table 1

Sums of rainfalls (mm) and mean air temperature (^oC) in 2004-2006 and in the long- term period 1971-1988 (research station Zamość)

Monts							
Years	Apr	May	June	July	Aug	Oct	Sum Apr-Oct
Precipitation (mm) – month sum							
2004	46.3	50.1	34.9	145.0	71.9	36.3	384.5
2005	45.4	98.2	69.5	33.6	52.7	15.8	315.2
2006	58.4	54.0	43.5	28.3	144.8	0.8	329.8
1971-1988	39.0	62.0	90.0	80.0	60.0	55.0	386.0
Temperature (^oC) – month mean							
2004	9.6	13.5	18.1	19.4	19.7	14.3	2890
2005	9.7	15.4	17.5	21.8	18.7	13.3	2948
2006	10.5	14.8	18.4	23.3	19.0	16.8	3144
1971-1988	7.2	13.4	15.8	17.4	16.8	12.6	2544

Experimental design and treatments

The experiment was carried out with two factors in a split plot design, with four replications. The first factor consisted of two treatments of fertilization: basic – NPK (A) and enriched NPK in S, Mg and micronutrients (B), while the second one comprised three medium-early potato cultivars: Irga (1), Mila (2) and Sante (3). The plot size was 30 m^2, the central part (19.5 m^2) of each plot was harvested. The row-space was 67.5 cm with 44.000 tubers ha^{-1} planted. Having harvested the forecrop, spring triticale, the straw was cut (5 $t \cdot ha^{-1}$) supplemented with 50 kg N in the form of urea (1 kg N per 100 kg straw) and winter rapeseed sown. In the third decade of March, the rapeseed was sprayed with 36% isopropylamine salt of glyphosate (Roundup 360 SL, 0.360 g a.i. kg^{-1} Monsanto) in dose 1080 g a.i. ha^{-1}. In the second decade of April, prior to the potato planting, the following two levels of mineral nutrition were applied:

A – basic (NPK): (kg ha^{-1}) N–100 (as urea), P_2O_5–90 (as mineral superphosphate), K_2O–140 (as potash salt),

B – enriched (NPK + SMg + micro-nutrients): ($kg \cdot ha^{-1}$) N–100 (as urea), P_2O_5–90 (as mineral superphosphate), K_2O–140 (as potash salt balanced with potassium sulphate), MgO–30 (as magnesium sulphate balanced with bitter-salt), S–25 (as potassium sulphate balanced with magnesium sulphate and with bitter-salt), INSOL-7-Potato (as N–14.0, B–0.50, Zn–1.5, Mn–1.5, Cu–0.50%) (2 l ha^{-1}).

The NPK fertilizers, partly S and Mg were applied before the potato planting. Bitter-salt and INSOL-7 – Potato mixed up with Miedzian 50 WP and Decis 2,5 EC were used for the foliar nutrition of potato plants at the growth stage determined by BBCH-scale: 49/55 (combined treatment for late blight and potato beetle control). Potato planters are not equipped with attachments to apply materials for *Rhizoctonia solani* control. The weed control was mechanical and chemical: from potato planting until emergence: harrowing, earthing up, weeding, while after emergence: herbicide metribuzin (Sencor 70 WP, 700 g a.i. kg^{-1}, Bayer) in dose 0.350 g a.i. ha^{-1}.

Fungicides for the control of *Phytophthora infestans* and other foliage diseases were applied four times:

1) oxadiksyl + mancozeb (Sandofan Manco 64 WP, 80 g a.i. kg^{-1} of oxadiksyl + 560 g a.i. kg^{-1} of mancozeb, Syngenta) in dose 160 g a.i. ha^{-1} + 1120 g a.i. ha^{-1};
2) copper (oxachloride form) (Miedzian 50 WP, 500 g a.i. kg^{-1}, Organika) in dose 1250 g a.i. ha^{-1}, 3. mancozeb (Dithane 75 WG 750 g a.i. kg^{-1}, Dow AgroSciences) in dose 1500 g a.i. ha^{-1};
4) fentin (hydroxide form) (Brestanid 502 SC., 502 g a.i. kg^{-1}, Aventis) in dose 251 g a.i. ha^{-1}.

The control of potato beetle (*Leptinotarsa decemlineata*) consisted of triple application of safe, inexpensive insecticides:

1) teflunenzuron (Nomolt 150 S.C., 150 g a.i. L^{-1}, BASF) in dose 37.5 g a.i. ha^{-1};
2) deltametryna (Decis 2,5 EC, 25 g a.i. L^{-1}, Aventis) in dose 7.5 g a.i. ha^{-1};
3) acetamiprid (Mospilan 20 SP, 200 g a.i. kg^{-1}, Nippon Soda) in dose 20 g a.i. ha^{-1}.

The chemical practices (the second and the third one) against late blight and chrysometids as well as fertilization feeding were performed jointly in order to reduce the number of crossings through the field (the preparations were mixed: Miedzian 50WP with Decis 2.5 EC with Bitter-salt and INSOL-7-Potato (2) and Dithane 75 WG with Mospilan 20 SP (3). The fourth protective treatment, a combined one, was carried out three weeks before the potato harvest. Brestanid 502 S.C. (for the protection of tubers against spores of potato blight) with dikwat desicant (in ion form) (Reglone Turbo 200 SL, 200 g s.a. L^{-1}, Syngenta) in dose 600 g s.a. ha^{-1}. Pesticides were applied by a 12-m-wide tractor-mounted sprayer that delivered 200 L ha^{-1} spray solution through 80-02 flat fan nozzles (model PILMET – P-412, Polen) at a spray pressure of 200 kPa.

The central part (19.5 m^2) of each plot was harvested in the second decade of September. From 2004 to 2006, in the 2nd decade of September, the tubers were visually rated for infection rate and infestation severity with *Streptomyces scabies* and *Rhizoctonia solani,* 1 to 9 scores corresponding to 0 to >25% and 0 to >50% infestation rate with *Rhizoctonia solani* and *Streptomyces scabies,* respectively. The visual score was carried out for a subset of samples (100 tubers or 10 kg, respectively) – Table 2 (ROZTROPOWICZ 1999).

Table 2

Scoring of infestations with Rhizoctonia solani and *Streptomyces* scabies on potato tubers (acc. ROZTROPOWICZ 1999)

Scoring	Infestation with *Streptomyces scabies* (percentage of scabs covering the tuber surface)	Infestation with *Rhizoctonia solani* (number/percentage of sclerotia covering the tuber surface)
9	none	none
8	< 5	< 10
7	6-10	~ 12
6	11-15	< 5%*
5	16-20	5 - 10%**
4	21-25	11 - 15%**
3	26-35	16 - 20%**
2	35-50	21 - 22%**
1	>50	>25%**

*small sclerotia; **small and big sclerotia

Statistical analysis

The results were analyzed statistically using the variance analysis with the F-Snedecor test function, then its distribution was computed (KLIKOCKA, SACHAJKO 2007). The significance of differences was indicated by Tukey«s HSD test at significance levels of $p<0.05$ and $p<0.01$. The coefficient of variation (*CV%*), a dispersion measure, was calculated as the quotient between the standard deviation (SD) and the mean. To establish the dependences and relationships between the elements studied, the analysis of correlation, determination and linear regression was applied (TRĘTOWSKI, WÓJCIK 1988). The comparison and summary of results was performed using Excel 7.0 worksheet and Statistica program (StatSoft Polska '97).

RESULTS AND DISCUSSION

The variance analysis revealed that differences in the potato tuber yields as well as *Streptomyces scabies* and *Rhizoctonia solani*–induced infection rates proved statistically significant.

The results given in Tables 3 and 4 show that the mineral fertilization levels (A–basic: NPK and B–enriched: NPK improved with S, Mg and microelements supplied as foliar and soil fertilizers) and the potato genotype type (1–Irga, 2–Mila, 3–Sante) influence the characteristics investigated. In this case, probability distribution of the F-test function at α=0.05 as well as dif-

Table 3

Results of statistical computation for investigated features

Investigated features (n=72, df_{error}=55	Variable	SED	CV%	Estimation F	p-value	LSD α=0.05*
Tuber yield (t ha^{-1})	F (df=1) C (df=2) FC (df=2) Y (df=2)	1.46 4.46 5.55 1.34	4.22 13.19 15.99 3.87	4.03* 42.80** 3.36* 2.26	0.049 6.2E-12 0.042 0.114	1.46 1.79/2.38 2.53 n.s.
Streptomyces scabies (infection rate %)	F C FC Y	1.42 3.40 4.20 3.00	8.77 23.95 25.91 18.54	4.86* 32.06** 0.81 14.46**	0.032 5.9E-10 0.450 8.9E-06	1.29 1.58/2.11 n.s. 1.58/2.11
Streptomyces scabies (infection severity 1-9)	F C FC Y	0.24 0.76 0.83 0.65	4.20 13.22 14.31 11.13	4.45 39.29** 1.57 20.84**	0.039 2.5E-11 0.217 1.8E-07	n.s. 0.28/0.38 n.s. 0.28/0.38
Rhizoctonia solani (infection rate %)	F C FC Y	0.92 0.88 1.12 0.66	24.07 22.10 29.17 17.15	53.67** 29.69** 9.13** 18.15**	1.1E-09 1.8E-09 3.8E-04 8.8E-07	0.25/0.34 0.31/0.41 0.44/0.58 0.31/0.41

Variable: F – fertilization treatments, C – cultivars, FC – fertilization treatments x cultivars, Y – years, df – degrees of variable freedom, df_{error} – degrees of error freedom, SED – standard error, CV% – coefficient of variation, estimation F of variance analysis: significant difference at (*α=0.05, ** α=0.01), p-value of F-variance ratio, LSD – significant difference, n.s. – not significant

ferences measured by Tukey«s test were significant. It was only potato tuber infection developed by *Streptomyces scabies* (scored 1-9) that appeared to be independent from the mineral fertilization. Generally, the analysed potato traits proved to be most highly varied (*CV%*) and differentiated (LSD) by the genotype (cultivar), then the mineral fertilization level and the cultivar response to fertilizers rather than the weather factor (Tables 3, 4).

The analysis of the research results confirmed a positive effect of the NPK fertilization improvement with sulphur, magnesium and Insol-7-Potato preparation (as N, B, Zn, Mn, Cu) – Table 3. NPK fertilization enriched with S and Mg and micronutrients in liquid fertilizer Insol 7 (B) increased potato tuber yield about 5.8% in comparison to NPK fertilization (A). The highest tuber yield was recorded for cv. Sante, which was justified by the genotype. The cultivars Sante and Irga responded in a highly positive manner to NPK fertilization (A), while the cultivar Mila exhibited a slight decrease in yield, although it was within the statistical error limits. Figure 1 shows the relationship between tuber yields of particular potato cultivars and tuber diseases (percentage infection rate) in dependence on fertilization. In plots where basic NPK fertilization (A) was used no significant relationship between tuber yield and tuber diseases occurred. Where enriched NPK

Table 4

Value of investigated features (means in years 2004-2006)

Investigated features (*n*=72)	Fertilization treatments (F) / / Year (Y)	Cultivars (C)			Mean
		Irga	Mila	Sante	
Tuber yield (t ha^{-1})	basic (A) enriched (B)	31.77 34.37	30.54 29.10	38.65 43.70	33.65 35.72
	2004 2005 2006	32.82 33.29 33.10	25.96 29.74 33.76	40.76 42.38 40.38	33.18 35.14 35.75
	mean	33.18	29.82	41.17	34.69
Streptomyces scabies (infection rate %)	basic (A) enriched (B)	22.58 19.28	15.85 15.37	13.17 10.92	17.20 15.19
	2004 2005 2006	17.24 23.61 21.93	11.00 18.75 17.07	10.00 12.38 13.77	12.75 18.25 17.59
	mean	20.93	15.61	12.05	16.19
Streptomyces scabies (infection severity 1-9)	basic (A) enriched (B)	4.64 5.16	5.94 5.87	6.38 6.97	5.65 6.00
	2004 2005 2006	5.71 4.21 4.78	6.85 5.36 5.51	7.16 6.63 6.24	6.57 5.40 5.51
	mean	4.90	5.91	6.68	5.83
Rhizoctonia solani (infection rate %)	basic (A) enriched (B)	5.84 3.47	3.33 2.63	4.26 3.42	4.48 3.17
	2004 2005 2006	5.52 4.53 3.93	3.93 3.60 1.41	3.44 4.19 3.89	4.29 4.10 3.08
	mean	4.66	2.98	3.84	3.82
*Rhizoctonia solan*i (infection severity 1-9)	basic (A) enriched (B)	5.60 6.61	7.12 7.48	6.76 7.16	6.49 7.08
	2004 2005 2006	5.73 6.32 6.27	6.95 6.57 8.38	7.02 7.10 6.76	6.57 6.66 7.13
	mean	6.11	7.30	6.96	6.79

fertilization with S and Mg and micronutrients (B) was used, the yield of potato tuber of cv. Mila was significantly negatively correlated (r = -0.62) and determined (R^2 = 37) to be infected by *Rhizoctonia solani*. In this case, enriched fertilization NPK (B) increased yield of tubers simultaneously decreasing infection of tubers by rhizoctoniose. SALAZAR (2006) reports that the fungus *Rhizoctonia solani* was found in Peru and Bolivia and attacks be-

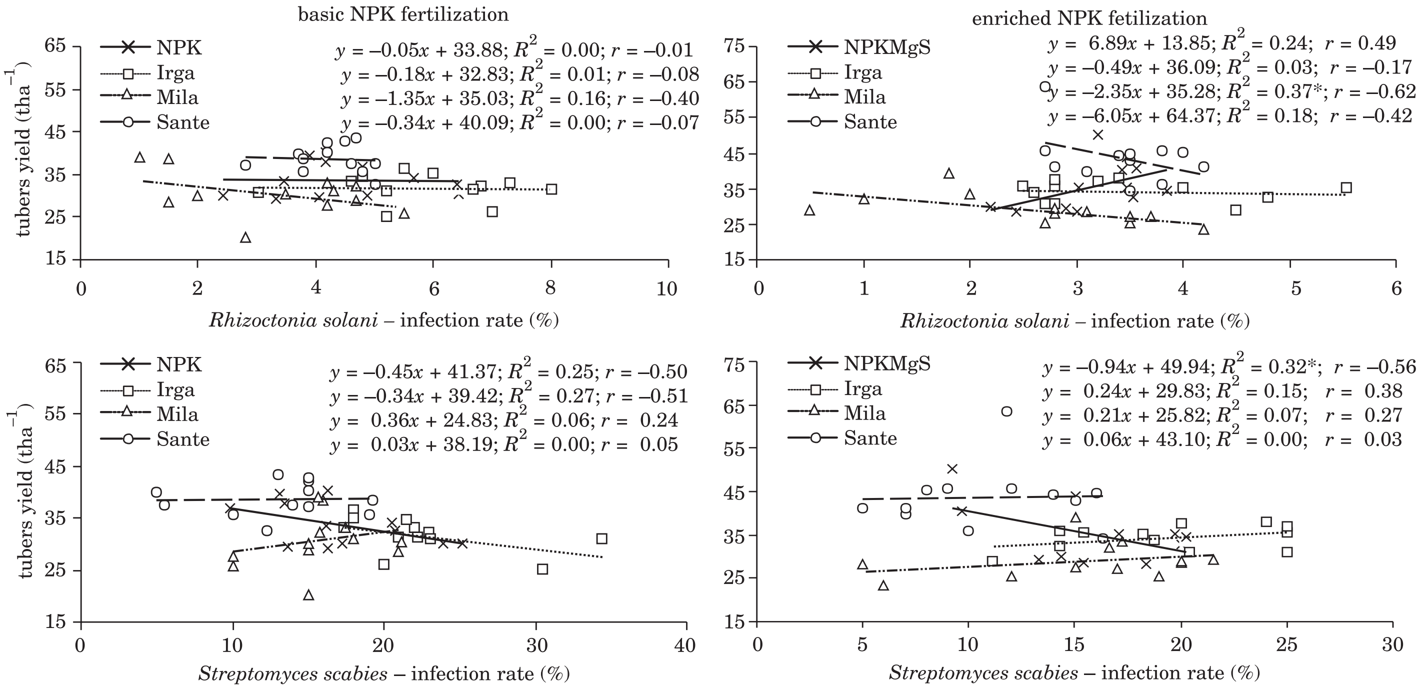

Fig. 1. The relationship between tuber yield and infection of tubers by Rhizoctonia solani and Streptomyces scabies

tween between 2 and 35% of tubers. Losses due to black scurf were estimated at 5 to 10%. In our experiment, when plots received enriched NPK fertilization (B) the tuber yield of all the three cultivars was significantly negatively correlated (r = -0.56) and determined (R^2 = 32%) to suffer from *Streptomyces scabies*-induced infection. Also, in this case enriched fertilization NPK (B) increased yield of tubers and decreased at the same time infection of tuber by scab. SALAZAR (2006) wrote that *S. scabies* occurred in Bolivia and incidences of infections varied between 28 to 40% causing yield losses up to 20% (Figure 1). LAMBERT et al. (2005) reports that nutrient effects could include alternations in the physiological or structural components of the plant's defense system, increased antagonistic microbial activity, or direct toxity to the pathogen. GRZEBISZ and HÄRDTER (2006) states that potato plants respond with high yielding and wholesomeness to sulphur and magnesium fertilization.

The mean infection rate of potato tubers by *Streptomyces scabies* reached 16.2%, whereas the mean infection severity (scored 1-9) was assessed at 5.83% (Table 4). The highest infection rate developed by this pathogen (percentage and 1-9 score) occurred on the NPK fertilized plots (A) On the objects with enriched NPK fertilization (B), incidence and infection severity of common scab-induced infection were significantly reduced: 11.7% and 5.8% (1-9 score). A statistically significant difference, though, has been confirmed only for infection rate. GROCHOLL and SCHEID (2002) as well as PICKNY and GROCHOLL (2002), who studied experimental plots fertilized with elemental sulphur and $CaSO_4$ did not find any direct relation between sulphur application and consistent reduction of potato tuber infection by *Streptomyces scabies*. Only a decreased soil pH value due to sulphur supplementation may enhance potato tuber resistance to this pathogen (GROCHOLL, SCHEID 2002, PAVLISTA 1995, PICKNY, GROCHOLL 2002). KLIKOCKA (2005)demonstrated that bringing soil pH down through sulphur dressing alleviated the symptoms of tuber infection by common scab by approximately 22%, and by 15% on the 1-9 scale. Some authors believe (GRZEBISZ, HÄRDTER 2006, ROGOZIŃSKA 1991) that magnesium supplementation, especially in the form of Kieresite, reduces potato tuber infection by common scab. Moderate N fertilization, at a well balanced N:P:K rate, e.g. 1:1:1.5, may suppress the pathogen development. Common scab is likely to be severe in soils of pH 5.5-8.0; in more acid soils the disease is reduced. Besides, a range of 13-15°C temperature under field conditions favours infection of potato tuber (FOTYMA, ZIĘBA 1988). Green manure ploughed into soil favours activation of microbes antagonistic to pathogenic strains of scab (BORÓWCZAK, GŁADYSIAK 1997). The cultivar Irga tubers were most susceptible to common scab: infection rate 20.93% and severity 4.90 (1-9 score). The tubers of cv. Mila and Sante proved to be less infected than those of cv. Irga: infection rate of 25.4% and 42.2% and infection severity 17.1 and 26.6% (1-9 score), respectively. The cultivar Mila responded weakly to enriched NPK fertilization (B) as no reduction in the infection rate developed by the pathogen was established. However, potato tubers of the cultivars Irga and Sante were significantly less infected by scab under NPK fertilization with sulphur, magnesium and microelements.

Generally, the infection of potato tubers by common scab was more strongly related to the cultivar (the cultivars Mila and Sante) than mineral fertilization. Also the weather conditions had n important effect because the variation for infection rate reached 18.54% and for severity 11.13% (1-9 score). SZUTKOWSKA (1998), GŁUSKA (2002) and LUTOMIRSKA (2002) report that high precipitation rate during the potato tuber formation period (especially in June) suppresses common scab incidence in potato tubers as rainfalls at that time is antagonistic to *S. scabies* bacteria penetrating soft potato skin. The highest susceptibility to infection was demonstrated by potato tubers during the stage of tuber initiation until the tuber diameter reached 1 cm. Therefore, the most efficient way to depress or even eradicate common scab disease is to keep soil moist during the active growth period (GŁUSKA 2002). Besides, a more severe *S. scabies*-induced infection of the cv. Mila tubers results from the widespread use of Sencor herbicide (metribusine) (KLIKOCKA 2000). Due to a great variability of the causal agents involved in the infection and development of this disease, no efficient common scab control strategy has been elaborated yet, so as to obtain plants free from this disease. Application of fungicides (comprising sulphur or its compounds) for the potato seed material dressing is highly recommendable. Fungicides have beneficial effects on potato plants, mainly in their early growth stage. However, they do not ensure complete protection against potato tuber infection induced by soil-borne *Streptomyces scabies*. in the later stages of the vegetation season. At that time, elemental sulphur should be implemented, which lowers the soil pH and may alleviate the symptoms of potato infection produced by this pathogen (KLIKOCKA 2005).

The mean infection rate of potato tubers by *Rhizoctonia solani* was 3.82%, while the mean infection severity (1-9 score) reached 6.79 (Table 3). In Germany, the highest tuber infestation by rhizoctonioza varied within 14-20% (KURZINGER 1995). The highest infection incidence in potato tubers produced by the pathogen was recorded in the samples obtained from the NPK fertilized plots (A). NPK fertilization improved with foliar and soil application of S, Mg and microelements (B) has significantly increased potato tuber wholesomeness in the three cultivars, reducing the disease incidence by 29.2% and severity by 8.3% (1-9 score). DATNOFF et al. (2007), HUBER, HANEKLAUS (2007), SALAC (2005), KLIKOCKA (2005) and HANEKLAUS et al. (2002) emphasize the preventive role of sulphur against fungal diseases. Beside chemical methods and cultivation of resistant varieties, agronomic practice is of the utmost importance for prevention of plant diseases. It has been shown that the mineral fertilization (with macro- and microelements) promotes natural plant resistance mechanisms, just like application of silicon, aluminium and sulphur (DATNOFF et al. 2007, KLIKOCKA et al. 2005, SALAC 2005, HANEKLAUS et al. 2002).

Potato tubers produced by cv. Irga appeared to be most severly infected by rhizoctonioza (infection incidence 4.66% and infection severity 6.11 (1-9 score). The cultivars Mila and Sante were healthier than the Irga (36.0 and 17.6% as expressed by infection rate and 16.3 and 12.2% by infection severity (1-9 score), respectively). On the whole, *R. solani* infestation of potato tubers varied subject mainly to the cultivar and mineral fertilization, while being less dependent on the climatic conditions, where the variability reached 18.15% for infection rate and 9.87% (1-9 score) for severity.

As high moisture and low soil temperature favour rhizoctoniose progression, the potato planting date is expected to be the optimal agrotechnical time concerning traits of a planted variety, soil conditions, climatic features of the region as well as regional weather forecasts (KLIKOCKA 2001). LUTOMIRSKA (2007) reported testing thirty two potato cultivars of various maturity groups for black scurf. The most important factor affecting disease symptoms was soil temperature in full growth of plants and 30 days before harvest. The temperature of air seems to be less important. There was no influence of rainfall on sclerotia occurrence. The cultivars differ in their susceptibility to *R. solani* development on tubers. SOWA-NIEDZIAŁKOWSKA and KRZYSZTOFIK (2008) state that the genotype (cultivars) is the main factor which affects losses by infection of tubers with diseases. Healthy potato seed material should be planted in which sclerotia infestation of tubers does not surpass 20% of the total surface (HÄNI et al. 1976). Besides, inappropriate forecrops like legumes, cruciferous plants and grasses should be avoided (PUŁA, ŁABZA 2007). KEMPENAAR and STRUIK (2007) reports that green-crop harvesting, i.e. vine removal and lifting, followed by a field period during which skin set can take place, can be very useful in controlling *Rhizoctonia solani*. Recent-

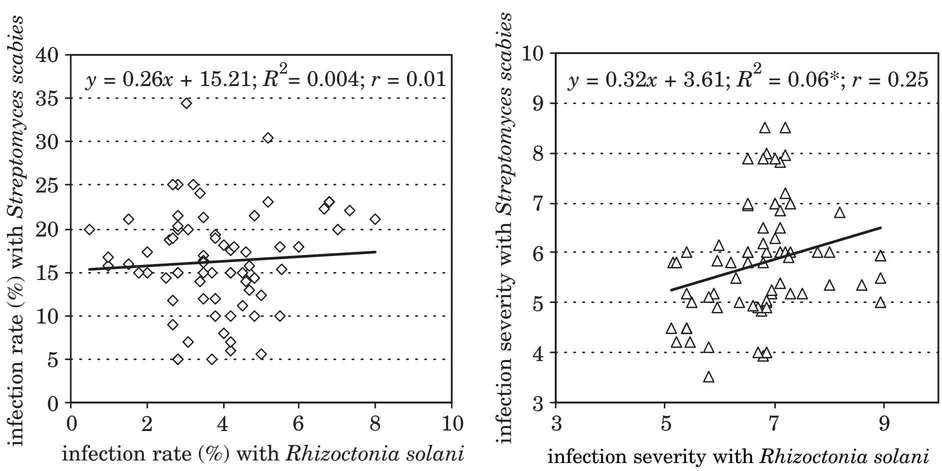

Fig. 2. Relationships between infection – rate and severity between *Rhizoctonia solani* and *Streptomyces scabies* on potato tubers

ly, efforts have been undertaken to use microorganisms (*Trichoderma viride, Bacillus sp.*) for potato protection against *Rhizoctonia solani* (KURZAWIŃSKA, GAJDA 2001). HAMM (2007) proposed control of soil-borne fungi (also *R. solani*) by the use of soil fumigants and/or seed treatments. Resistance in newly developed potato cultivars shows a promise for controlling many of these plant pathogens and will be the principal mechanism for controlling soil-borne fungi and fungal like organisms in the future.

Significant relationships between the infection severity of *Rhizoctonia solani* versus *Steptomyces scabies* on potato tubers were found (Figure 2). The results presented in Figure 2 reveal that the infestation of the tubers with both pathogens run linearly and any measure improving the natural resistance of the plant will supposedly combat both pathogens.

CONCLUSIONS

1. The potato characteristics analyzed (skin infection of tuber, yield) proved to be mostly affected and differentiated by the genotype (variety), less so by mineral fertilization and its interaction with a variety. That has been confirmed by the statistical analysis performed.

2. Enriched fertilization – B (NPK supplemented by soil and foliar application of sulphur, magnesium and microelements) has significantly reduced potato tuber infection by *Streptomyces scabies* for the cultivars Irga and Sante. Each potato cultivar responded in a different way to infection by this pathogen, the most severe infestation by common scab was demonstrated by the cultivar Irga, significantly less infected were the cultivars Mila and Sante; the cv. Mila did not react positively to enriched fertilization (B). Enriched fertilization decreased significantly tuber infection by *Rhizoctonia solani* of all the three potato cultivars. The cultivars Mila and Sante proved to be healthiest, whereas the tubers of cv. Irga were more infected with rhizoctoniose.

3. Both diseases are interrelated since generation of one of them induces development of the other, therefore sulphur, magnesium and microelement dressing may control the activity of both pathogens.

4. Potato tuber yield was significantly higher after application of enriched fertilization (B) as compared to basic fertilization (A). The highest tuber crop was recorded for the cultivars Sante, which is justified by its genotype.

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THE EFFECT OF LATERAL ELECTRICAL SURFACE STIMULATION (LESS) ON SPINAL DEFORMITY IN IDIOPATHIC SCOLIOSIS*

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Abstract

Clinical studies were carried out in the period of 2003-2008 at the Provincial Children's Rehabilitation Hospital in Ameryka near Olsztyn (Poland). The study involved a group of children and youths exhibiting spinal deformity progression in idiopathic scoliosis (IS) of more than 5^{o} per year according to the Cobb scale. Four hundred and fifty patients between 4 and 15 years of age were divided into three groups (n = 150). Group I received 2-hour and group II 9-hour treatment of Lateral Electrical Surface Stimulation (LESS), respectively, whereas group III (control) was treated only with corrective exercises for 30 minutes twice a day. LESS was performed as 24-month treatment with the use of a battery-operated SCOL-2 stimulator manufactured by Elmech, Warsaw, Poland.

The effectiveness of this method was confirmed in the treatment of spinal IS in children and adolescent patients, especially when the initial spinal deformity does not exceed 20^{o} according to the Cobb scale. A short-duration electrostimulation – 2 hours daily – was

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found to produce results similar to those obtained after overnight, 9-hour electrostimulation. Moreover, the analysis of the Harrington prognostic index F confirms the positive effect of LESS in both groups of patients (2 h and 9 h of LESS).

Key words: electrostimulation, LESS, rehabilitation, idiopathic scoliosis.

WPŁYW BOCZNEJ ELEKTRYCZNEJ POWIERZCHNIOWEJ ELEKTROSTYMULACJI (LESS) NA DEFORMACJĘ KRĘGOSŁUPA W SKOLIOZIE IDIOPATYCZNEJ

Abstrakt

Badania kliniczne prowadzono w latach 2003-2008, w Wojewódzkim Szpitalu Rehabilitacyjnym dla Dzieci w Ameryce k. Olsztyna, w grupie dzieci i młodzieży wykazującej progresję skrzywienia w skoliozie idiopatycznej (SI) powyżej 5° wg Cobba w skali rocznej. Badaniami objęto 450 pacjentów, w wieku od 4 do 15 lat, podzielonych na 3 grupy ($n = 150$). W grupie I stosowano 2-godzinną terapię metodą Bocznej Elektrycznej Powierzchniowej Elektrostymulacji (Lateral Electrical Surface Electrostimulation – LESS), w grupie II – 9-godzinną, a w grupie III (kontrolnej) – tylko leczenie za pomocą ćwiczeń korekcyjnych wykonywanych 2 razy po 30 min dziennie. Elektrostymulację typu LESS stosowano, w 24-miesięcznym leczeniu za pomocą bateryjnego stymulatora SCOL-2, wytwarzanego w zakładach elektromechaniki medycznej Elmech w Warszawie.

Efektywność tej metody oceniano podczas leczenia SI kręgosłupa u dzieci i młodzieży, zwłaszcza w przypadkach, gdy początkowe skrzywienie kręgosłupa nie przekraczało 20° wg metody Cobba. Elektrostymulacja LESS skrócona do 2-godzinnej terapii dziennie wywoływała podobnie korzystne rezultaty do osiąganych podczas elektrostymulacji całonocnej – 9-godzinnej. Także wykonana analiza współczynnika prognostycznego F Harringtona potwierdziła pozytywne efekty oddziaływania metody LESS w obydwu grupach pacjentów (I i II).

Słowa kluczowe: elektrostymulacja, LESS, rehabilitacja, skolioza idiopatyczna.

INTRODUCTION

Idiopathic scoliosis (IS) in children and adolescents is an important problem for treatment centres due to its quite high prevalence. It has been estimated that globally this problem, depending on the method and the region, concerns from 0.3 to 15.3% of the population. In Poland, the incidence of this condition ranges from 2 to 14%, while in Western Europe it involves 2 to 3 % of the population. The majority (80-90%) of reported spinal deformities are those of idiopathic scoliosis (IS), i.e. arising from unknown cause (AHN et al. 2002, CHEN 2003, KOWALSKI 2004).

A wide variety of kinesitherapeutical methods applied in spinal lateral deformity (SLD) treatment have an undeniable effect stopping progression of the deformity, although current procedures are influenced by the interpretation of the pathogenesis of this condition (AHN et al. 2002, CHEN 2003, KOWALSKI 2004).

Based on electromyographic studies, a decreased activity at the concave side has been observed in the superficial muscles stabilizing the spine. Excessive activity of the convex side indicates a defensive response so as to maintain the spine in an upright position. This indicates an asymmetric activity of the muscles stabilising the spine (HIRAYAMA et al. 2001, WRIGHT et al. 1992).

However, asymmetric exercises have long been shown to be ineffective because certain spinal muscles, especially the intervertebral muscles, are not amenable to exercises aimed at their selective strengthening or loosening (DURMAŁA et al. 2003).

In recent years, attention has been paid to the disorders of the nervous-muscular system and their secondary effect on lesions in the spinal osteoarticular-ligament system as one of the potential causes of IS (KOWALSKI 2003, KOWALSKI et al. 2001, 2004). Therefore, LESS was introduced into the conservative treatment of IS.

The aim of this study was to determine the effectiveness of LESS therapy in treating children and youths with IS.

MATERIAL AND METHODS

Clinical studies, part of a research project financed by the State Committee for Scientific Research, were carried out in the years 2003-2008 at the Provincial Children's Rehabilitation Hospital in Ameryka near Olsztyn. The study involved a group of children with the progression of the spinal deformity of more than 5^o per year according to the Cobb method. In all the cases examined, scoliotic deformity was located within the thoracic segment of the spine between Th4 and L1. The Cobb deformity angle in the children examined ranged from 10^o to 39^o. The study involved 450 children and teenagers between 4 and 15 years of age divided into three groups (n = 150): group I and group II with 2-hour and 9-hour LESS, respectively, and group III – a control group.

Children in both stimulated (LESS) groups had additional corrective exercises at home, applied twice daily for 30 minutes, while children in group III were only treated with the same corrective exercises.

Electrical stimulation was performed with the use of a battery-operated SCOL-2 stimulator produced by Elmech, Warsaw (KOWALSKI, 2003 KOWALSKI et al., 2004, 2001, 2004). The stimulator's technical parameters were as follows: rectangular approx. 0.1 ms impulses and frequency of 20-55 Hz, duration of the impulse series of 3.5-4.5 s, impulse series intervals of 4-12 s, and the stimulation current amplitude range of 5-75 mA. The impulse waveforms during laboratory trials were recorded on a TEKTRONIX oscilloscope using a state-of-the-art technique for imaging electric signals.

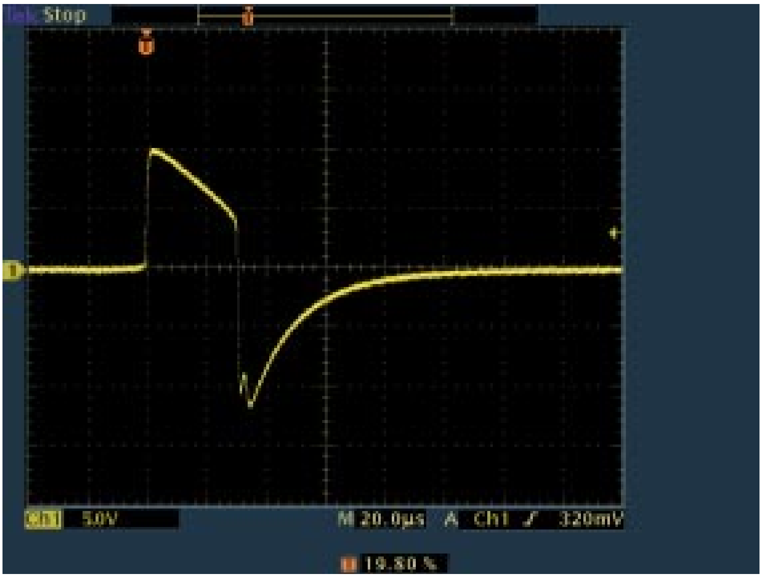


Fig. 1. Plot of minimum impulse amplitude set to 5 of scale

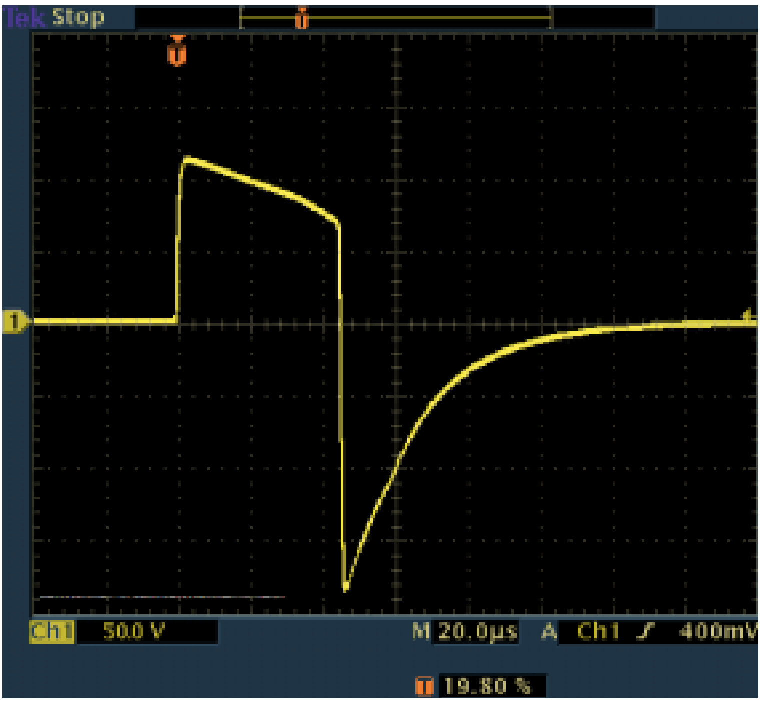


Fig. 2. Plot of maximum impulse amplitude set to 30 of scale

Impulses generated by the stimulator have the form of slightly differentiated rectangles, registered in a stimulator using a substitute resistance of 5.1 kΩ, similar to human tissue resistance (Figures 1, 2).

Electrical stimulation of trunk muscles was performed with superficial disc-shaped electrodes approximately 20 mm in diameter. The conductive rubber electrodes were placed along the scoliotic deformity every 6-12 cm, depending on its length, which is on average 10 cm.

It is recommended that the electrode be located below the blade of the shoulder blade alongside the spinal line at the convex side of the scoliotic deformity. There are three possible locations of the electrodes: medial, intermediate and lateral. Based on preliminary studies, the intermediate location ensures the best conditions of stimulation. In this location, the effect of LESS, both after long-duration (9 h) and short-duration (2 h) electrostimulation, was analysed in all cases.

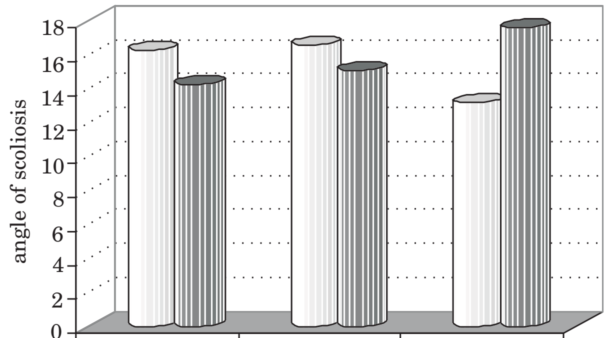
The effectiveness of the LESS treatment was evaluated from the measurements of the initial spinal deformity angle prior to the treatment and that after 24-month treatment (including corrective exercises in combination with stimulation or only corrective exercises in the control group). The deformity angle was measured in an upright position in an a-p projection according to the Cobb method in three subranges of angle value: 10-19°, 20-29° and 30-39°. The mean value of the deformity angle before treatment and the mean deformity decrease or scoliosis progression after treatment were determined for each experimental group. Moreover, the Harrington prognostic co-efficient F, defined as equal to the Cobb deformity angle divided by the number of vertebrae within the deformity, was determined.

All clinical results were statistically analysed with *t*-Student test.

RESULTS

The clinical results obtained after therapy with LESS (Figure 3) depended on the deformity angle range to which the patients were qualified before treatment. In the 10-19° range, some improvement after treatment was observed: on average 2° decrease in deformity angle size. This value is statistically significant at p=0.01. The LESS treatment proved to have a significantly positive effect in 75.5% of all cases of small deformities. In the 20 - 29° range, only a slight (0.3°) improvement in the scoliosis angle was observed after treatment. Stabilisation was observed in almost half of the patients, whereas in the 30-39° range a mean 1.4° scoliosis progression (not statistically significant) was recorded after treatment. Therefore, the therapeutic effect in this group of patients was small.

In the group of scolioses treated with LESS, the best therapeutic effect was obtained in patients with an initial spinal scoliosis angle below 20° according to Cobb method.



	LESS 2 h	LESS 9 h	control group
before treatment	16.3	16.6	13.2
after treatment	14.3	15.1	17.6

Fig. 3. Mean values of the deformity angle before and after treatment

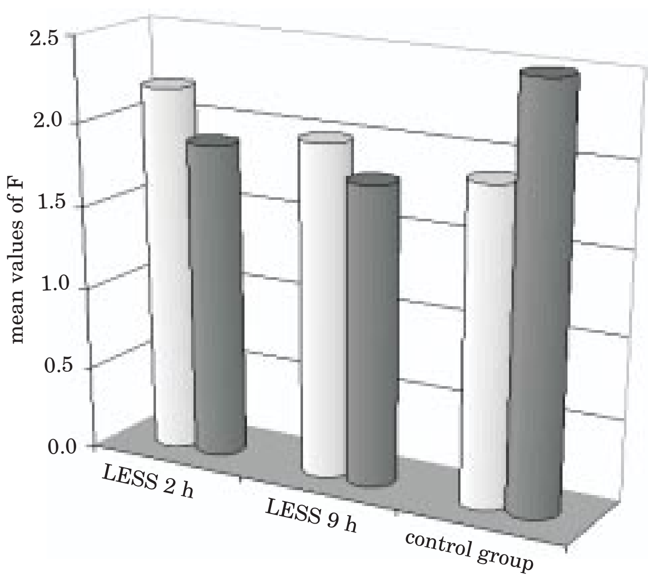


Fig. 4. Mean values of the F co-efficient before and after treatment

Moreover, the analysis of the Harrington prognostic index F confirms the positive effect of LESS in both groups of patients 2 h and 9 h of LESS treatment (Figure 4).

In the control group, scoliosis progression of approximately 4^{o} according to Cobb method (statistically significant at p=0.01) was observed despite 24-month rehabilitation.

DISCUSSION

Currently, functional electrostimulation of the nervous-muscular system (FES) generates positive effects. An ever-growing number of applications of electrostimulation as a supportive factor in other treatment methods has been observed. Results of studies carried out at different centres justify the application of LESS in the treatment of IS (KOWALSKI et al. 2004, ANCIAUX et al. 1991, AXELGAARD et al. 1983, BOBECHKO et al. 1979). Electrostimulation of spinal muscles is the only method which can protect asymmetric activity of spinal muscles including intervertebral muscles. Therefore, the non-invasive LESS method will hopefully be used in the treatment of children with IS (KOWALSKI et al. 2004, ANCIAUX et al. 1991, AXELGAARD et al. 1983, BOBECHKO et al. 1979). It can, however, only be used in grade I^{o} scoliosis, where there are no profound morphological lesions and no degenerative changes in concave side muscles (Anciaux et al. 1991, AXELGAARD et al. 1983, BOBECHKO et al. 1979).

BOBECHKO et al. (1979) was first (1974) to introduce electrostimulation for treating IS using superficial electrodes and then electrodes implanted into perispinal muscles. By implanting electrodes he wanted to reach deep layers of segmental muscles. Stimulation was performed in a horizontal position while sleeping, and lasted from 8 to 10 h. In 1983, Axelgaard (AXELGAARD al. 1983) showed that percutaneous electrostimulation with electrodes placed on the patient's back also produced advantageous results compared with those obtained with implanted electrodes. An increase in muscle tension can be obtained by direct stimulation of a muscle or by a reflex arc as well as by excitation causing facilitation of or decrease in inhibiting excitation (WRIGHT et al. 1992).

The results obtained by BOBECHKO et al. (1979) were encouraging enough for many authors to introduce electrostimulation among the methods used in treating spinal lateral scolioses. The main goal of LESS is to replace the non-existing or reinforce the defective bioelectrical activity with adequately formed series of electrical impulses acting directly on the affected nervous-muscular structures. An advantage of electrostimulation is the excitation of afferent nervous fibres through which the spinal cord structures are excited. Thus a substitute excitation model is initiated or a new stereotype of muscle activity control system is created (WRIGHT et al. 1992, BOBECHKO et al. 1979).

Over the last twenty years there have been many reports on the effects of LESS functional electrostimulation on reducing or stopping progression of the disease, or even a decrease in the initial spinal scoliosis. The results concerning the effectiveness of LESS are unequivocal especially as regards the range of the angular deformity for which this type of therapy could be used without causing side effects (KOWALSKI et al. 2004, ANCIAUX et al. 1991, AXELGAARD et al. 1983, BOBECHKO et al. 1979, BUCIŃSKI et al. 2004).

Weiss and Paśniczek were first in Poland to apply electrostimulation (WEISS et al. 1983, PAŚNICZEK et al. 2005). At the Konstancin Rehabilitation Centre (1979-80) they used electrostimulation to excite the perispinal muscles at the convex side of grade II° scoliosis in children between 11 and 14 years of age during scoliosis progression. The stimulations were performed according to an empirically established programme which included 3-month cyclic stimulations lasting 8 to 10 hours carried out during sleep. The results were positive: spinal scoliosis diminished after treatment, which was confirmed by radiological examinations. However, long-term results of the improvement have not been analysed (WEISS et al. 1983, PAŚNICZEK et al. 2005, ZARZYCKI et al. 1991).

In this paper an attempt has been made to prove that the LESS method of treating IS (KOWALSKI 2003, KOWALSKI et al. 2004, 2001, 2004) produces similar or even better results than traditional procedures. The results of the research which has been carried out by KOWALSKI since 1986 (partly presented in this paper) on a large group of children with IS prove that LESS has a good effect on the reduction or stabilisation of scoliosis. Based on these studies, the best treatment can be obtained when the initial scoliosis is less than 20°, measured according to Cobb method. KOWALSKI also found that the inconvenience of overnight therapy could be reduced by proposing a 2-h evening therapy with LESS, which is equally effective as an all-night 8-h stimulation (KOWALSKI 2003, KOWALSKI et al. 2004).

CONCLUSION

1. The analysed LESS method has a good effect on the nervous-muscular system in the therapy of children and youths with IS.

2. LESS has been found to be an effective method in treating IS, especially in cases where the initial spinal scoliosis is less than 20° according to Cobb method.

3. Short-duration electrostimulation (2h daily) produces similarly positive effects to those obtained after all night electrostimulation (9h) and eliminates undesirable symptoms of all night LESS therapy.

4. The analysis of the Harrington prognostic co-efficient F confirms the positive effect of LESS in both groups of patients (2 h and 9 h of LESS).

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BIOLOGICAL ASPECTS OF CADMIUM AND LEAD UPTAKE BY *PHRAGMITES AUSTRALIS* (CAV. TRIN EX STEUDEL) IN NATURAL WATER ECOSYSTEMS

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Abstract

In natural environment plants are exposed to many different stress factors, including heavy metals, whose elevated concentration causes oxidative stress, connected with formation of reactive oxygen species (ROS). Therefore, plants have developed defence systems, including enzymatic antioxidant system, able to remove ROS.

The work concerns the accumulation of two heavy metals, cadmium (Cd) and lead (Pb), as well as the phenomenon of oxidative stress caused by increased concentration of these metals in common reed (*Phragmites australis*), a dominant species in the littoral zone of many water reservoirs. The plants were obtained from four water bodies situated in Poznan: Kierskie Lake, Rusałka Lake, Strzeszyńskie Lake and Sołacki Pond.

The aim of the study was to examined the accumulation of heavy metals and the relation between activity of antioxidant enzymes in rhizome, stem and reed leaves during the vegetative period. Three antioxidant enzymes were analyzed: ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and superoxide dismutase (SOD).

The statistical analysis was done to determine the influence of the heavy metals on the activity of the antioxidant enzymes, involved in limiting and removing results of oxidative stress.

Heavy metals were accumulated in common reed in all the four water reservoirs, but the activity of enzymes was variable during the observation period. Statistical analyses suggest that there are some correlations among concentration of metals and the activity of antioxidative enzymes. However, the results do not provide an unambiguous determination of the effect of heavy metals on enzymatic activity. Summing up, the contamination

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of the water ecosystems caused by heavy metals was so low that it did not influence the activity of the analysed enzymes.

Key words: cadmium, lead, antioxidant enzymes, water reservoirs, bottom sediments, common reed *Phragmites australis*

BIOLOGICZNE ASPEKTY POBORU KADMU I OŁOWIU PRZEZ *PHRAGMITES AUSTRALIS* (CAV. TRIN EX STEUDEL) W NATURALNYCH ZBIORNIKACH WODNYCH

Abstrakt

W środowisku naturalnym rośliny są narażone na działanie metali ciężkich. Nadmierne ich stężenia w roślinach powodują stres oksydacyjny, wywoływany powstawaniem reaktywnych form tlenu (ROS). W celach obronnych organizmy roślinne wykształciły wiele systemów antyoksydacyjnych, w tym enzymatyczne umożliwiające usuwanie ROS.

Praca dotyczy akumulacji dwóch metali ciężkich – kadmu (Cd) i ołowiu (Pb) oraz zjawiska stresu oksydacyjnego w roślinach powodowanego przez nadmierne stężenia jonów metali ciężkich w środowisku oraz w organach trzciny pospolitej (*Phragmites australis*) – gatunku występującego i dominującego w strefie litoralnej wielu zbiorników. Materiał roślinny pochodził z czterech zbiorników wodnych zlokalizowanych w obrębie miasta Poznania: Jeziora Kierskiego, jeziora Rusałka, Jeziora Strzeszyńskiego i Stawu Sołackiego.

Celem badań było określenie zależności między zawartością metali ciężkich a aktywnością enzymów antyoksydacyjnych w kłączach, łodydze i liściach trzciny na przestrzeni okresu wegetacyjnego. Analizowano aktywność trzech enzymów antyoksydacyjnych: peroksydazy askorbinianowej (APX) i gwajakolowej (GPX) oraz dysmutazy ponadtlenkowej (SOD).

Przeprowadzono analizy statystyczne celem określenia wpływu czynników stresu oksydacyjnego wywołanego przez metale ciężkie na aktywność enzymów antyoksydacyjnych, które są zaangażowane w ograniczanie i usuwanie skutków tego zjawiska.

Metale ciężkie akumulowane w roślinach we wszystkich ocenianych zbiornikach wodnych wykazywały tendencję wzrostową, natomiast aktywność enzymów w roślinach była silnie zróżnicowana w całym okresie obserwacji. Analizy statystyczne sugerują wprawdzie istnienie pewnych korelacji między stężeniem metali w roślinach a aktywnością enzymów antyoksydacyjnych, jednakże nie pozwala to na jednoznaczne określenie wpływu badanych metali na zmienność w aktywności tych enzymów. Podsumowując, stwierdzone skażenie ekosystemów wodnych metalami ciężkimi było na tyle niskie, że nie wpływało modyfikująco na aktywność badanych enzymów.

Słowa kluczowe: kadm, ołów, enzymy antyoksydacyjne, zbiorniki wodne, osady denne, trzcina pospolita (*Phragmites australis*).

INTRODUCTION

Water reservoirs are very attractive components of open and urban landscapes, but in order to serve their functions well they need to have good water quality. The quality of water in surface water bodies is largely determined by the concentration of heavy metals. Stability of heavy metals in aquatic environment and their accumulation in successive links of the food chain make it absolutely necessary to search for efficient and safe methods to remove these toxic elements from individual elements of the ecosystem, especially sediments and plants inhabiting water bodies.

Although the total content of heavy metals in the environment is very low, less than 1%, and while some of the metals are vital for living organisms, cadmium and lead are examples of toxic elements. Heavy metals enter natural ecosystems with discharges from industry and urban sewage or through atmospheric deposition, which increases their concentration in nature. Most of them remain insoluble in soil and water ecosystems or, as dissolved elements, become available for plant uptake and cumulate in their tissues (GRZYBOWSKI et al. 2000). Plant uptake directly reduces the overflow of metals into adjacent waters (LIANG et al. 2006). The accumulation in plants and the direction and intensity of transport depend on biological factors and on the chemical properties of metals.

Cadmium and lead belong to pollutants present in areas with heavy road traffic and near cities, which are taken by plants but at different bioaccumulation indexes. Generally, the highest Pb concentrations have been observed in roots, while only small amounts are transported to other parts of plants (VERMA, DUBEY 2003). In contrast, cadmium belongs to the elements capable of being translocated through plants and accumulated in leaves. However, in the experiments conducted by Fediuc and Erdei (2002) on young *Phragmites australis* grown hydroponically, most of the Cd content was collected in the shoots.

Some plants, called hyperaccumulators, such as *Thlaspi caerulenscens* and *Brassica juncea*, demonstrate a particularly high ability to accumulate metals in unusually high concentrations (SALT et al. 1998). Also common reed (*Phragmites australis*), which is a widely distributed species worldwide, proves useful in cleaning eutrophic lakes and waste waters (YE et al. 1997).

High concentrations of cadmium or lead can also be stress factors for plants (IANELLI et al. 2002, VERMA, DUBEY 2003). They can disrupt cellular homeostasis and enhance the generation of reactive oxygen intermediates (ROIs), which cause oxidative stress. To avoid ROIs, plants create mechanisms called ROI-scavenging mechanisms, which are responsible for removing reactive oxygen intermediates (e.g. singlet oxygen (O_2^1), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) (MITTLER 2002). The scavenging systems include antioxidant enzymes: catalase (EC 1.11.1.6), peroxidases (EC 1.11.1.7), superoxide dismutases (EC 1.15.1.1), and low molecular antioxidants such as ascorbic acid, glutathione or a-tocopherol (MITTLER 2002, VERMA, DUBEY 2003).

The aim of the study was to examine the relation between the level of heavy metals in sediments of natural water ecosystems (four lakes near the city of Poznan, Poland) and their accumulation in *Phragmites australis* plants growing in those reservoirs. The study focused on cadmium and lead bioaccumulation and the interrelations between those contaminants in aquatic plants and the activity of antioxidant defence enzymes (which can be indicators of stress caused by toxic metals), such as ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and superoxide dismutase (SOD), including the effect of seasons.

MATERIALS AND METHODS

Study area and plant material

Four recreational water reservoirs located in the city of Poznań were selected for the study: Lake Kierskie (referred to as KL), Lake Strzeszyńskie (SL), Lake Rusałka (RL) and Sołacki Pond (SP). The experiments were performed in 2005 and 2006. Four heavy metals, i.e. zinc, cadmium, lead and copper, were determined in the bottom deposits and in whole plants of common reed (*Phragmites australis*, Cav. Trin. ex Steudel). Independently, cadmium and lead concentrations and the antioxidant enzymes were analysed separately in leaves, stems and rhizomes (without adventitious roots). All analyses were carried out three times during the vegetation period, i.e. in May, August, and November. Plants, after being transferred to the laboratory, were weighed and frozen in liquid nitrogen.

Heavy metal analyses

Plant samples (1 g) and sediments (3 g) were dried at 105°C for 48 h and mineralised in a Star 6 microwave oven (CEM) for 40 minutes, adding 25 cm^3 HNO_3 and 5 cm^3 H_2O_2. Metal contents were estimated by electrothermal atomic absorption spectroscopy using an AAS spectrometer (Varian Spectra AA 200 Plus).

Antioxidant enzyme analyses

For ascorbate and guaiacol peroxidase (APX and GPX) frozen samples (0.2 g) were homogenized for 30 seconds in a chilled mortar with 50 mM phosphate potassium-buffer (pH 7.0) and 2% Polyclar AT. Homogenates were centrifuged at 15 000 g for 30 minutes at 4°C. APX activity was determined according to Nakano and Asada (1981). The reaction mixture contained 50 mM phosphate-potassium buffer, 0.5 mM L-ascorbate, 0.1 mM H_2O_2 and enzyme extract. Absorbance was measured at λ_{max} = 290 nm using an UV/VIS Spectrophotometer Lambda 11 (Perkin Elmer) and was expressed as absorbance increment per 1 minute per 1 mg protein ($\Delta E\ min^{-1}\ mg^{-1}$).

GPX activity was measured according to HAMMERSCHMIT et al. (1982). Enzyme assays contained 25 mM phosphate-potassium buffer (pH 7.0), 0.2 mM guaiacol, 0.09 mM H_2O_2 and enzyme extract. Absorbance was recorded at λ_{max} = 480 nm and was expressed as absorbance increment per 1 minute per 1 mg protein ($\Delta E\ min^{-1}\ mg^{-1}$).

Superoxide dismutase (SOD) was assayed according to Beauchamp and Fridovich (1971). Frozen tissues (0.2 g) were homogenized in a chilled mortar with 50 mM phosphate-potassium buffer (pH 7.8), containing 1% polyvinylpolypyrrolidone (PVP), 1.0 mM EDTA-Na and 0.5 M NaCl. Extracts were centrifuged at 15 000 g for 25 min at 4°C. The incubation mixture contained 50 mM phosphate-potassium buffer (pH 7.8), 0.01 mM EDTA-Na, 4 mM methionine, 0.1 mM nitro blue tetrazolium (NBT) and crude extract. Finally, 2.4 mM riboflavin was added and afterwards samples were placed under an UV lamp for 10 minutes. At the same time a blank sample was prepared. Absorbance was measured, in relation to the blank test, at λ_{max} = 560 nm using an UV/VIS Spectrophotometer Lambda 11 (Perkin Elmer). One unit of SOD activity was defined as the quantity of the enzyme required to reduce absorbance by 50% in comparison to the blank sample per one mg of protein. The level of proteins was measured according to Bradford (1976) using serum albumin (Sigma) as a standard.

Statistical analysis

All preparations, for each year, were done as independent triplicates. Standard deviations for means were calculated and correlations and linear regressions were determined using Statistica and GraphPad Prim software.

Multivariate analysis of variance for factorial experiments was applied in order to determine spatial and time variability in metal contents (zinc, cadmium, lead and copper) in sediments of analyzed reservoirs and in *Phragmites australis* plants.

RESULTS

Estimation of heavy metal contamination in water reservoirs

The analysis of canonical variables, used to assess the variation and similarity of analyzed reservoirs in terms of heavy metal accumulation in sediments, showed that the biggest differences in the contents of analyzed metals in sediments were found between Sołacki Pond (SP) and the other reservoirs (Figure 1*a*). Mahalanobis distances between this reservoir and the other water bodies were 230.95, 186.46 and 207.64 for KL, SL and RL, respectively, and they were on average 4 times bigger than that e.g. between KL and SL, amounting to 54.27. Thus, a markedly different distribution of metals was recorded in the SP sediment, which may be related to the location of this reservoir closest to the city centre of Poznań.

Graphic analysis of similarities and differences in metal contents in sediments in terms of sampling dates showed the dissimilarity of the third date, i.e. autumn (November) – Figure 1*b*. Metal contents at that time were

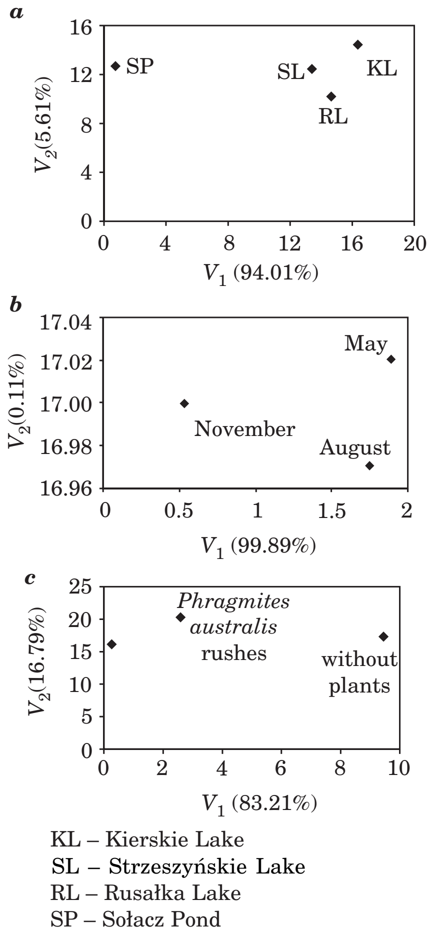

Fig. 1 . Similarities of heavy metal accumulation in sediments analyzed in years 2005-2006: *a* – between four reservoirs (KL, SL, RL and SP), *b* – between periods of analyses, *c* – rushes compare to area out of plants

higher than on the other two dates, which is confirmed by the calculated Mahalanobis distance, which for May and November was 10 times higher than for May and August.

When analyzing rushes, i.e. sites overgrown with shore plants, in terms of the accumulation of heavy metals in sediments, differences were found between sampling sites in the case of samples free from plants and sites where macrophytes were found, which indicates that heavy metals are accumulated in aquatic macrophytes (Figure 1*c*). The discussed differences are reflected in the calculated Mahalanobis distance, which amounted to 70.04 for *Phragmites australis* rushes and a site free from vegetation.

Cadmium and lead accumulation in common reed

Annual mean cadmium content in common reed organs was 2-fold higher in 2006 than in 2005 (Figure 2*a*). The lowest concentration was recorded in plants collected from Lake Kierskie (KL), a slightly higher level was observed in those from Lake Strzeszynskie (SL) and the highest – in plants from Rusałka (RL) and Sołacki Pond (SP). Similar relations were observed in both years.

The average lead concentration was 5-10-fold higher than that of cadmium and similar in both years. The lowest accumulation was recorded in plants collected from Lake Strzeszyńskie (SL). In the remaining reservoirs concentrations of this metal were similar.

When analyzing similarities of plant collection dates in the years 2005-2006 in terms of the accumulation of all the analyzed metals (Zn, Cd, Pb and Cu), a considerable difference was found between the first (May) and the third date (November) (Figure 2*b*).

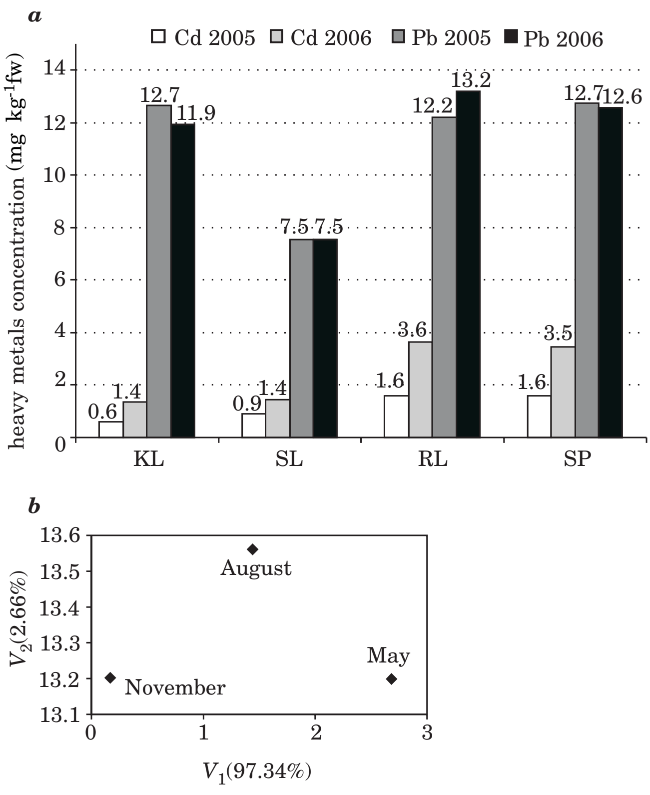

Fig. 2. Heavy metal accumulation in common reed (*Phragmites australis*) grown in four natural water ecosystems (KL, SL, RL and SP – explanations see Fig. 1) analyzed in 2005-2006: *a* – cadmium and lead concentration, *b* – similarity between periods of analyses (means for zinc, cadmium, lead and copper)

Moreover, in 2005 the accumulation of both metals was analysed in individual organs, i.e. in leaves, stems and rhizomes. In all plant parts the lowest content of cadmium (Figure 3) was recorded in May, at the beginning of the vegetative period, and the highest – at the end of vegetation (November), so the Cd content was increasing during the growth process. There was a noticeable disproportion in its accumulation depending on the examined lakes: slight in KL and SL, while considerable in RL and SP plants.

The level of lead contamination measured in leaves was similar in all the lakes and changed little during the vegetation season (Figure 4). In shoots its content was twice as high in KL as in the other lakes and

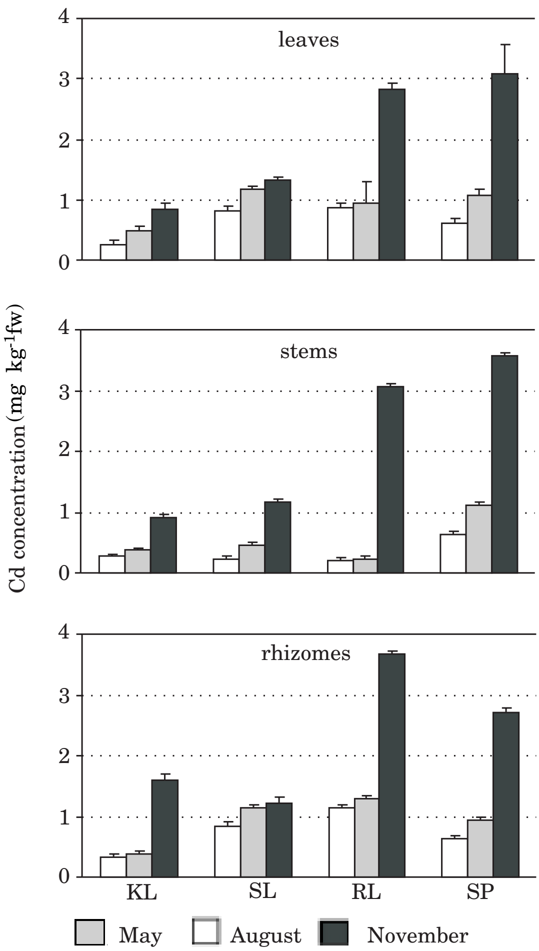

Fig. 3. Cadmium concentration in common reed leaves, stems and rhizomes grown in natural water ecosystems (KL, SL, RL, SP – explanations see Fig. 1), measured in May, August, and November

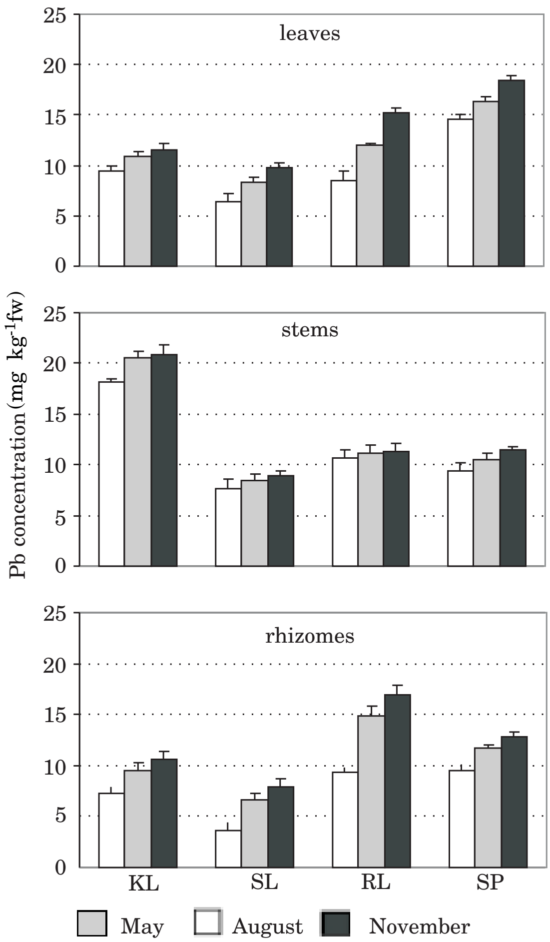

Fig. 4. Lead concentration in common reed leaves, stems and rhizomes grown in natural water ecosystems (KL, SL, RL, SP– explanations see Fig. 1), measured in May, August, and November

during the vegetation period the changes were noticeable. In rhizomes the Pb content increased between May and November and was relatively similar in plants from all the lakes.

Table 1 presents bioaccumulation indexes of elements, expressed in the ratios of contents in leaves and rhizomes to the concentrations of this metal in the bottom sediment of the reservoir. The value of this index recorded for cadmium was 2- to 4-fold higher than that of lead. When comparing the bioaccumulation index of both analyzed metals in aerial and underground parts of common reed plants, higher bioaccumulation of Cd and Pb was found in rhizomes.

Table 1

Bioaccumulation of cadmium and lead in *Phragmites australis* measured in leaves and rhizomes in relation to sediment (mean for 2005 and 2006)

Metal	Plant organ	Bioaccumulation index			
		KL	SL	RL	SP
Cd	leaves	2.91	0.91	1.13	1.26
	rhizomes	4.62	1.90	1.28	0.96
Pb	leaves	1.01	0.93	0.81	0.36
	rhizomes	1.28	1.31	1.03	0.30

KL, SL, RL, SP – see Fig 1.

In conclusion, Pb content in common reed tissues exceeded the level of Cd and its dynamics of changes in individual plant organs and bioaccumulation indexes were different for the examined lakes.

Antioxidant enzyme activity

The constitutive level of ascorbate peroxidase (APX) activity was the highest in leaves and the lowest in common reed stems (Figure 5*a*). During the experimental season the changes were negligible in leaves and stems. In rhizomes the APX level was very low in May and then successively increased, irrespective of the analyzed water reservoir.

Guaiacol peroxidase (GPX) activity was relatively high in common reed stems as compared to leaves and rhizomes (Figure 5*b)*. The changes were not dependent on the investigated water ecosystem.

Superoxide dismutase (SOD) activity was the highest at the beginning of plant growth, especially in leaves (Figure 5*c*). In stems and rhizomes the highest activities were observed in the middle of the summer (August). Generally, no differences were found between the analysed lakes.

Statistical analysis

In order to compare heavy metal accumulation and antioxidant enzyme activities, correlation coefficients were calculated using the Statistica software. In 2005 all data were subjected to analyses jointly, without division into individual plant organs and lakes. A positive correlation was found between lead concentration and the activity of ascorbate (r=0.423 at p<0.05) and guaiacol peroxidases (r=0.42 at p<0.05). A negative correlation was observed between the concentration of cadmium and the activity of superoxide dismutase (Table 2).

In 2006 analyses were carried out for individual organs and some effect was observed only for leaves. Correlations between the concentration of cadmium and GPX activity (r=-0.35 at p<0.05) and lead and both enzymes (r=-0.31 and r=-0.45) were negative.

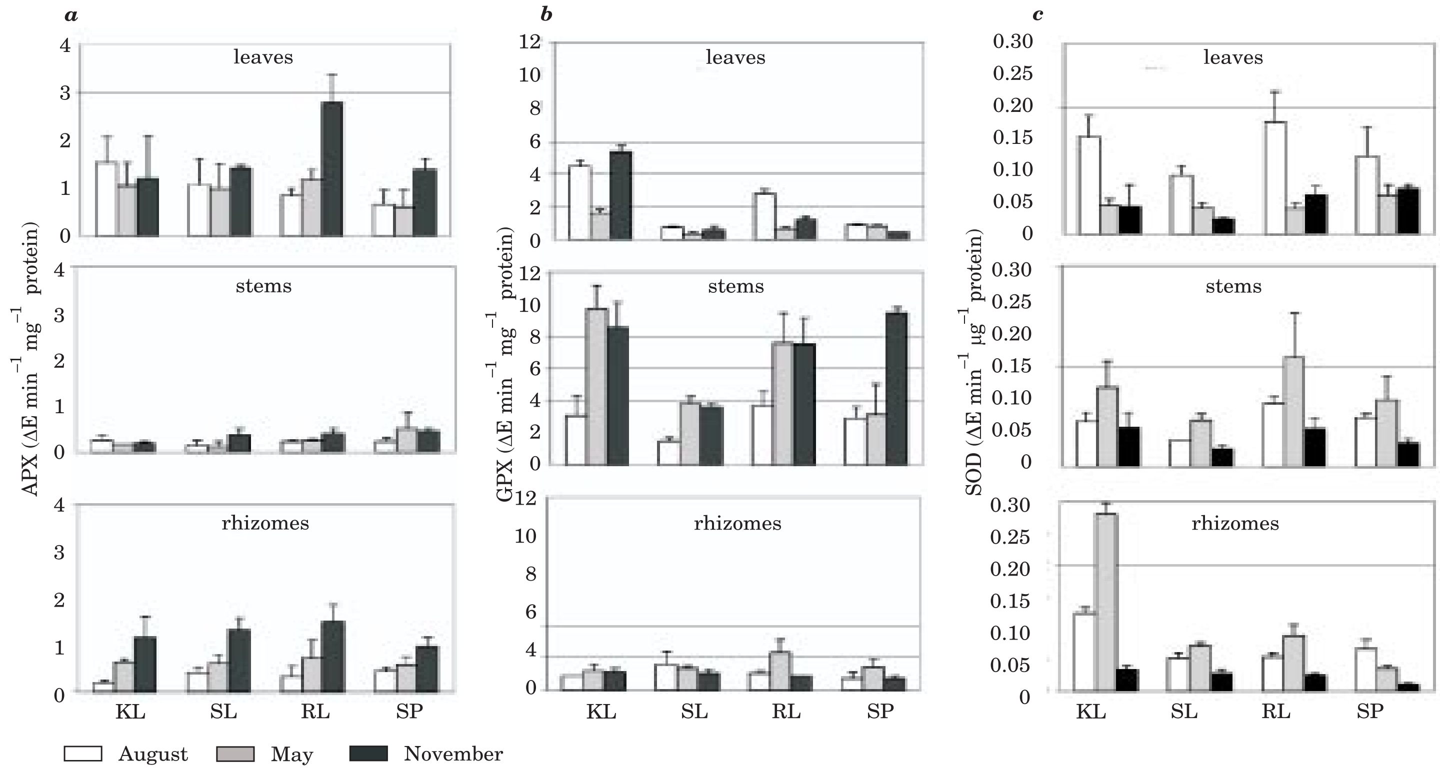

Fig. 5. Activity of antioxidant enzymes APX, GPX and SOD in common reed leaves, stems and rhizomes grown in natural water ecosystems (KL, SL, RL, SP), measured in May, August and November

Table 2

Correlation coefficient for linear regression between cadmium and lead accumulation and the enzymatic activity

Metal accumulation	Enzyme activity		
	APX	POD	SOD
Results of 2005 (whole plants)			
Cd	0.088 –	0.004 –	- 0.538 +
Pb	0.423 +	0.420 +	- 0.069 –
Results of 2006 (leaves):			
Cd	0.17 –	- 0.35 +	
Pb	- 0.31 +	- 0.45 +	

\+ linear dependence
– linear independence

DISCUSSION

The examined water reservoirs are classified as typical urban reservoirs, in which the quality of water is affected by the neighbouring Poznan agglomeration. When analyzing sediments from the water reservoirs, similar contents of the four analyzed heavy metals were determined in threelakes: KL, SL and RL. Moreover, contamination of bottom sediments was statistically higher in the autumn period (November) than in the spring-summer period. In turn, the uptake of metals by *Ph. australis* plants was most intensive in the spring-summer period, which was probably caused both by intensive transpiration and the demand for functional minerals. Heavy metals are also absorbed with nutrients. In the late autumn, especially in 2005, low temperature promoting the transition of plant to the state of dormancy reduced metal uptake, even at their highest content in sediments. This dependence was not observed in the case of accumulation of cadmium and lead.

Contents of these contaminants, especially toxic metals, increase from May until November, as has been reported by YE (1997) and VERMA and DUBEY (2003).

The data concerning bioaccumulation of cadmium and lead are not conclusive. However, cadmium is considered to be an element of a much higher mobility than lead, although its content in the environment is much lower, which was also confirmed in our study. In the analyzed water reservoirs, Cd content was at least several-fold lower than that of Pb and varied in successive years of the study. In terms of the metal content in the analyzed organs, accumulation of both Cd and Pb adjusted to the fresh weight basis was similar. In model studies using small *Ph. australis* plants generated from embryonic callus tissue, roots accumulated more cadmium than shoots, which would suggest limited mobility of Cd in this species (FEDIUC, ERDEI 2002). SIMILARLY, YE et al. (1997) stated that the highest content of cadmium was accumulated in reed roots. These data, similarly as the results of our investigations, indicate that this is characteristic for this aquatic plant. Another species, *Typha latifolia*, under identical experimental conditions accumulated three times more cadmium than *Ph. australis* and higher concentrations were recorded in shoots when compared to common reed.

The fact that lead was accumulated mostly in the underground parts of common reed is consistent with the commonly accepted view and observations on different plant species (WINDHAM 2001). However, the content of heavy metals in plants is dependent on their concentration in the nutrition solution, in soil or in water ecosystems, etc. (YE 1997, WINDHAM 2001, VERMA DUBEY 2003).

An important part of this study was an attempt to assess the dependence between the degree of reservoir contamination, bioaccumulation of elements, especially cadmium and lead, and the activity of antioxidant enzymes. A positive correlation between antioxidant enzyme activities and heavy metals was recorded only between guaiacol and ascorbate peroxidases and lead, although changes in the content of this element were slight. Such correlations are known from literature data, but pertain to cadmium (FEDIUC et al. 2001, IANNELI et al. 2002, RULEY et al. 2004, PACZKOWSKA et al. 2007). A negative correlation was recorded between superoxide dismutase and cadmium, which was very different from results available in published data, e.g. SHAH and KUMAR (2001) presented opposite results for rice.

Different results and few statistically proven relations may have been caused by the fact that plants were taken directly from their natural environment, where concentrations of cadmium and lead were considerably lower than in plants used in laboratory experiments. Furthermore, various environmental factors, such as temperature or solar exposure, etc., had a considerable effect on the activity of antioxidant enzymes.

CONCLUSION

Very low concentrations of heavy metals in the natural ecosystems were determined in the present study, with variations occurring between the reservoirs and between the sampling dates (the spring and autumn periods).

Although marked cadmium bioaccumulation was recorded, it did not have an effect on the activity of antioxidant enzymes. Enzymatic activities could have been stimulated by other environmental factors. At such a low heavy metal contamination, the tolerance of *Ph. australis* is probably sufficient for homeostasis.

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ESTIMATION OF ABSORPTION OF MAGNESIUM NICOTINATE AND ITS DERIVATIVES WITH SELECTED AMINO ACIDS

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Abstract

Preparation of solid dispersions is a popular pharmaceutical technology designed to improve the solubility and absorption characteristics of drugs. Solubilizing and moisturizing of carriers show influence on therapeutic substances; although dissolution of molecular dispersion of particles of the therapeutic substance in a neutral carrier is of utmost importance.

This paper present the results of the research on influence of modification the structure of magnesium nicotinate Mg(Nic) with ligands, glycine and arginine, on the absorption process of Mg^{2+}ions *in vitro.* The absorption area was the small intestine of a rat. It was found that structural changes with an additional arginine or glycine ligand affect the absorption process of Mg^{2+}ions.

Moreover, the effect of hydrophilic carriers on the partition coefficient (log P) for the system of n-octanol and phosphate buffer was investigated for the solid dispersions containing the examined magnesium salts. Phosphatidylcholine (PC-45) and polyvinylpirrolidone (PVP K-30) were used as carriers for solid dispersions with of magnesium salts. It was confirmed that using auxiliary substances PC-45 and PVP changes significantly ($p<0.05$) P values, corresponding to increasing hydrophobic properties of solid dispersions of the examined salts.

It was found that modification of the structure of magnesium nicotinate by amino acids such as arginine or glycine positively influences the absorption process Mg^{2+} ions. The research carried out on properties of the solid dispersions containing magnesium salts and phospatidylcholine (PC-45) or magnesium salts and polyvinylpirrolidone (PVP K30) showed positive influence of these auxiliary substances.

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Key words: magnesium nicotinate, ligands: glycine, arginine, solid dispersion, partition coefficient, absorption.

OCENA WCHŁANIANIA NIKOTYNIANU MAGNEZOWEGO I JEGO POCHODNYCH Z WYBRANYMI AMINOKWASAMI

Abstrakt

Wytwarzanie stałych dyspersji jest popularną metodą technologiczną stosowaną w celu poprawy rozpuszczalności i wchłaniania leków. Właściwości solubilizujące oraz zwilżające nośników mają wpływ na proces rozpuszczania substancji leczniczych.

W pracy przedstawiono badania wpływu modyfikacji struktury nikotynianu magnezowego Mg(Nik) aminokwasami (argininą lub glicyną) na proces wchłaniania jonów Mg^{2+} *in vitro*. Powierzchnię absorpcji stanowiło jelito cienkie szczura. Stwierdzono, że dodatkowy ligand argininy lub glicyny w strukturze nikotynanu magnezu wpływa na zmianę parametrów procesu wchłaniania jonów Mg^{2+}.

Ponadto badano wpływ nośników hydrofilowych na współczynnik podziału o/w układu *n*-oktanol/bufor fosforanowy wybranych soli magnezowych stałych rozproszeń. Do sporządzenia stałych rozproszeń z badanymi solami magnezowymi zastosowano fosfatydylocholinę 45% (PC-45) i polivinylopirolidon (PVP K-30).

Stwierdzono, że zastosowanie substancji pomocniczych PC-45, PVP znacząco ($p<0.05$) wpływa na zmianę wartości log P, a zatem wzrasta hydrofobowość stałych rozproszeń badanych soli. Wykazano, że modyfikacja struktury nikotynianu magnezu argininą lub glicyną wpływa na poprawę absorpcji jonów magnezowych. Badania właściwości stałych rozproszeń zawierających sole magnezowe – fosfatydylocholinę (PC-45) lub sole magnezowe – poliwinylopirolidon (PVP K-30) wykazały pozytywny wpływ zastosowanych substancji pomocniczych.

Słowa kluczowe: nikotynian magnezu, ligandy: glicyna, arginina, stałe rozproszenia, współczynnik podziału, wchłanianie.

INTRODUCTION

Magnesium belongs to essential macroelements which condition proper functions of human body. It takes part in all important metabolic changes, in reactions of synthesis of energetically rich compounds mainly ATP, hydrogen and electron carriers, or in synthesis and activity of numerous enzymes. It plays an important role in oxidation-reduction processes or maintaining acid-basic balance. Magnesium has also been demonstrated to alleviate stress, allergies, anaphylactic condition or inflammation states and to participate in defence processes (Szmitz et al. 2007, Touyz 2004). Magnesium is a stabilizer of cell membranes, influencing their fluidity and permeability (Konrad et al. 2004). Based on numerous studies it has been shown that Mg chelated with amino acids and pyridoxine is well absorbed. It has been proved that amino acids such as aspartic acid, cysteine, arginine and glycine act as carriers in Mg^{2+} ion transport (Olędzka 1999, Marcoin and Szulc 2002).

Magnesium is essential in human diet and its lack can cause many diseases (GRIGORYAN, KOMPANTSEVA 2005). Oral or intravenous Mg supplementation is an effective method of combating and preventing its deficiency (SKIBNIEWSKA 2000). Market demand for preparations containing their very important bioelement is continually growing.

For supplementation the best are magnesium compounds containing organic anions, chelate compounds of moderate strength, which protect metal ions against binding in sparingly soluble compounds (FIROZ, GRABER 2001) They facilitate its transport through walls of intestines and release metal ions into serum, where they can be added to right receptors and then transmitted to cells. While working on new drugs, mechanisms and factors conditioning gastrointestinal absorption are of the utmost importance. Hydrophobicity, a property indicating ability of a drug to permeate through cell membranes, has great importance for the interaction of the drug with a receptor. Hydrophobicity of a given compound can be determined experimentally by finding a partition coefficient between the two phases (log P), a parameter determining lipophilic-hydrophilic balance, which reflects passive transport of a drug in organism.

Preparations containing nicotinic acid (pyridino-3-carboxylic acid) with magnesium are applied in disturbances of peripheral circulation treatment, hypercholesteremy, migraine, podagra. Mineral amino acids chelate (glycinate nicotinate: Cu, Zn, Cr, Ca, Mg) are applied to supplement the diet its source of bioelements found in food meant for people.

In order to obtain a drug with improved Mg^{2+} ions absorption, modification of the structure of a magnesium nicotinate molecule was attained via amino acids ligands. In the previous paper (MARCOIN and SZULC 2002), positive influence of an additional ligand of glycine to the structure of magnesium nicotinate in the process of absorption of Mg^{2+} ions in the small intestine was presented.

The subject of this paper is comparative evaluation of *in vitro* absorption of Mg^{2+} ions from magnesium nicotinate and magnesium nicotinate modified with arginine or glycine. Increasing dissolution rate of therapeutic substances in solid dispersions depends on the kind and amount of the carrier as well as the production method. Production of solid dispersions is among methods frequently used in order to improve pharmaceutical availability and, consequently, bioavailability of therapeutic substances. The most common carriers to produce solid dispersions are polyvinylpyrrolidone (PVP), polyethylenglycols (PEG), derivatives of cellulose and phospholipids. Solubilizing and moisturizing properties of carriers show influence on dissolution process of therapeutic substances; yet molecular dispersion of particles of a therapeutic substance in a neutral carrier has the greatest significance.

MATERIALS AND METHODS

The following salts were examined:

- magnesium nicotinate: Mg(Nic), $Mg(C_6H_4 O_2N)_2$, mol.wt. 268.31
- magnesium aginine-nicotinate: Mg(NicArg), $Mg(C_{12}H_{18}O_4N_5)$, mol.wt. 320.43
- magnesium glycine-nicotinate: Mg(NicGly), $Mg(C_8H_8O_4N_2)$, mol.wt. 284.31.

In order to produce solid dispersions the auxiliary substances such as:

- phosphatidylcholine 45% (PC-45), (Lucas Meyer, Ltd);
- polivinylopirolidon (PVP), (Serva), were used.

All the chemicals were analytical reagent grade.

The synthesis of magnesium nicotinate was carried out according to the procedure described in the paper of MARCOIN and RYSZKA (1991). The magnesium nicotinate salts with amino acids (arginine or glycine) were obtained in a reaction of magnesium nicotinate and an appropriate amino acid in water solution of molar ratio 1:1. The synthesis was carried out at 60-70°C, the mixture being stirred intensely for 3 h. The products of the synthesis were isolated from the solution by water evaporation under low pressure evaporator (Unipam – 350), then crystallized from methanol and dried at room temperature. The content of magnesium was measured in an atomic absorption spectrophotometer (Carl Zeiss Jena model AAF 3) at the wavelength of 285.2 nm.

Preparation of solid dispersions. Solid dispersions were prepared in the granule form. Micronized magnesium salt was mixed with the selected carrier (PC-45 or PVP) in molar ratio (1:10) and dissolved in ethanol. After complete evaporation of ethanol, the solid dispersions were dried under vacuum and unified through a sieve (1.0 mm).

*Partition coefficient o/w (*log P*)*. For the solid dispersions the partition coefficient o/w (log P) for the system of n-octanol/phosphate buffer was determined according to the theory delineated by HANSCH et al. (1962).

Absorption process of Mg^{2+} *ions in vitro for magnesium salts.* Investigation of the absorption process of Mg^{2+} ions for magnesium salts was carried on an *in vitro* model according to the method described previously (MARCOIN AND SZULC 2002), in which the absorption area was the small intestine (ileum) of a rat. The essential part of this apparatus was a glass chamber of 30 cm^3 capacity, thermostated at 37°C and filled with solution of 4 mM of the analysed magnesium salt. Aqueous 0.9% NaCl solution was pumped with a peristaltic pump through the intestine segment at a constant collected rate of 1.2 ml min^{-1}. Samples were collected every 15 min. and the magnesium content was measured by atomic absorption spectrophotometry (Spectrophotometer of AAF 3 Carl Zeis Jena) at the wavelength 285.2 nm. The study had been approved by the Bioethics Committee of the Medical University of Silesia. The results consisting of the absorption rate constant (k) and absorption half time ($t_{50\%}$) were calculated. The measurements were repeated six times in order to minimise statistical errors. Standard deviation (SD) and variance (V) were determined.

Statistical analysis. All values were expressed as mean ± SEM. The measurements were repeated six times. Statistical significance was tested by repeated measures using ANOVA followed by Kruskal-Wallis test or esle Post Hoc multiple comparisons were done. $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Modification of the magnesium nicotinate structure with ligand of arginine or glycine had positive influence on the parameters describing kinetics of Mg^{2+} ionic absorption in the small intestine of a *in vitro* rat system. The results are presented in Table 1 and Figure 1. Absorption of Mg^{2+} ions in a segment of the small intestine was carried out in agreement with the first order kinetics. The absorption of Mg^{2+} ions was the fastest for Mg(NicArg), followed by Mg(NicGly), but it was the slowest for the parent compound Mg(Nic). The amount of absorbed Mg^{2+} ions for all the measurement points showed statistically significant differences ($p<0.05$) compared with Mg(Nic) (Figure 1). Significant difference was found between Mg(Nic) and Mg(NicGly) ($p<0.05$) after 75 and 90 minutes.

Comparing the half time of absorption ($t_{50\%}$) of Mg^{2+} ions with magnesium nicotinate modified by arginine with the parent compound shows its decrease of 0.75 hour. The parameters specifying the absorption process indicate that arginine and glycine are good carriers for transporting Mg^{2+} ions. Analysis of the present results shows that structural changes with an

Table 1

Parameters describing Mg^{2+}-ions absorption from magnesium salts in a small intestine

salts	$k*10^{-3}$ (min)	$t_{50\%}$ (h)	Total amount (%) of absorbed Mg^{2+} ions within 2 h of the experiment	V (%)	(±) SD
Mg (Nic)	1.99	6.04	19.80	5.31	1.04
Mg (NicArg)	2.20*	5.29*	22.51*	3.88	0.87
Mg (NicGly)	2.07	5.58	21.40	5.48	1.15

*$P<0.05$ vs. Mg(Nic), k – absorption rate constant; $t_{50\%}$ – absorption half -time; V – variance; SD – standard deviation

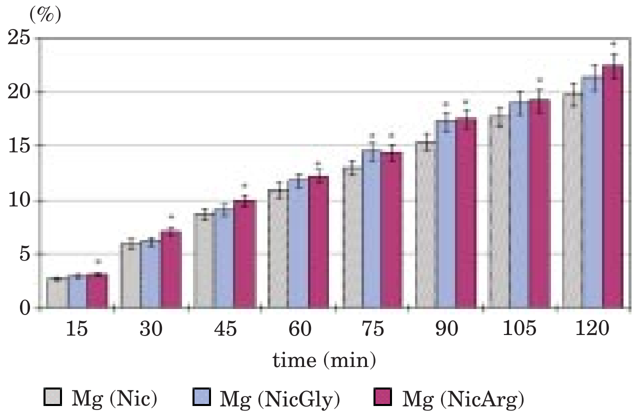

Fig. 1. The absorption rates of Mg2+ through the rat's small intestine from solutions of magnesium salts with modified structure.
Values are expressed as mean ± SD (n=6). *P<0.05 vs. Mg(Nic) at the same time point

Table 1

Parameters describing Mg^{2+}-ions absorption from magnesium salts in a small intestine

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V – variance; SD – standard deviation

additional arginine or glycine ligand modify absorption of Mg^{2+}. Increasing the length of a magnesium nicotinate chain by adding ligand positively influences polarity, which is the sum of various intermolecular reactions of donor – acceptor type.

In the case of magnesium nicotinate modified with glycine ligand, the factors characteristic for magnesium absorption were improved. Comparing the effects of amino acids applied to modify magnesium nicotinate, the influence of the substituent structure is evident. By introducing appropriate substituents into a particle, it is possible to influence its physicochemical properties and, eventually, its biological activity. The donor atoms such as oxygen and nitrogen found in particles of amino acids condition formation of bindings. The guanidine group may form characteristic pairs of dionic hydrogen bonds, which are responsible for the power of bonding. It is well known that the guanidine group of arginyl radicals is important for proteins to maintain the tertiary protein structure through interior "salt bridges" with carboxyl groups and for bonding and differentiating amino substrates by enzymes, receptor sites (LEHN 1985).

Reactions with amino acids may alter molecule properties such as solubility, partition coefficient between n-octanol phase and water one as well as other characteristics which are of importance for drug absorption, distribution and excretion. Particles capable of reacting with anions by means of hydrogen bonds through electrically neutral polar centers (e.g. hydroxyl, amide groups) become a class of potential carriers making diffusion via membranes easier (MARGALIT et al. 1979).

Figure 2 contains the log P values determined for the partition coefficient of the examined dispersions containing selected magnesium salts between n-octanol phase and water phase. As the calculated values of the partition coefficient (log P) for solid dispersions without addition of auxiliary substances such as PVP and PC-45 show, modification of the parent compound with glycine ligand increases log P value by 0.149 units ($p<0.05$) while for the modification with arginine ligand the increase is 0.090 ($p<0.05$). Using auxiliary substances such as PVP, PC-45 influences significantly ($p<0.05$) change of log P values, therefore hydrophobicity of the examined solid dispersions containing magnesium salts increases. Addition of PC-45 is more effective than that of PVP for solid dispersions containing Mg(NicArg) and

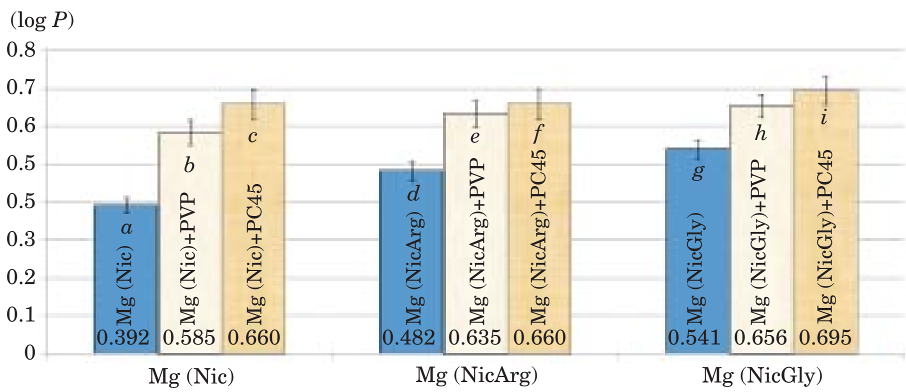

Fig. 2. The values partition coefficient (log P) for solid dispersions containing magnesium salts. Values are expressed as mean ± SD (n=6).

d,g bars : $^*P<0.05$ vs. *a* bar; *b,c* bars: $^*P<0.05$ vs. *a* bar; *e,f* bars: $^*P<0.05$ vs. *d* bar; *h,i* bars: $^*P<0.05$ vs. *g* bar.

Magnesium salts: Mg(Nic), Mg(NicArg), Mg(NicGly). Solid dispersions: Mg(Nic)+PVP, Mg(Nic)+PC45, Mg(NicArg)+PVP,
Mg(NicArg)+PC45, Mg(NicGly)+PVP, Mg(NicGly)+PC45

Mg(NicGly). The highest log P value (0.695) was obtained in the case of Mg(NicGly); for Mg(NicArg) the log P value equalled 0.660. The improvement of hydrophobic properties of the magnesium salts contained in the solid dispersions via addition of PVP and PC45 carriers depends on their physicochemical properties.

Phosphatidylcholine conditions the hydrophobic balance and creates hydrogen bonds with a drug. Phosphatidylcholine was applied as a carrier in dispersion systems for indomethacin, phenobarbital, benzodiazepine derivatives (LAW et al. 1992). Complex studies of solid dispersions proved that the applied carriers can improve the rate of dissolution and, consequently, pharmaceutical availability of drugs. Positive results were obtained by MARSAC et al. (2008) who examined solid dispersions containing nifedipine and felodipine in the presence of PVP. Physical stability of the examined dispersions was linked with their amorphic properties as well as low hygroscopic properties.

PATEL and PATEL (2007) obtained improvement of lovastatin solubility for PVP polymer matrix dispersion. They identified decrease of the crystalline and increase of the amorphous fraction of the drug by means of X-ray, DSC,FT-IR analysis methods. DHUMAL et al.(2007), in examinations of stability of solid oral forms of a drug containing celecoxib, used PVP and carrageenan. They did not find recrystallization of amorphous drugs since preparation, during processing and further storage.

Positive influence of water soluble polymers (PVP) on hydroxypropyl-beta-cyclodextrin complexation of rofecoxib was described by SINGH and ABOUL-ENEIN (2007). Considerable improvement of pharmaceutical availability of valdecoxib was attained by application of solid dispersions with PVP in tablets (AFTAB and PRALHAD 2006). In order to improve solubility of tenoxicam and flurinazine EL- GAZAYERLY et al. (2000) and MARIN et al. (2002) used PVP as a carrier for these substances in solid dispersions.

CONCLUSION

Modification of magnesium nicotinate structure with arginine or glycine ligand influences both solubility and ability of Mg^{2+} ions to penetrate through the small intestine of a rat. Additional ligands of arginine or glycine in the magnesium nicotinate structure are said to be good carriers of Mg^{2+} ions. The use of the auxiliary substances such as PVP, PC-45 in solid dispersions causes a decrease of dissolution and absorption process, which is evidenced by log P values. Addition of PC-45 to solid dispersion is more advantageous than PVP.

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EFFECT OF UREA APPLIED WITH COMPOSTS ON CONCENTRATION OF Cu, Zn AND Mn IN CORN FRESH MATTER

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Abstract

Corn was grown for green matter in a pot experiment, in which soil was fertilized with composts alone or in combination with 3 g N per pot (urea). The composts were made in wooden boxes, measuring 50×60×60 cm. They were composted for 3.5 months, until the temperature in the composts became stable and equal to the ambient temperature. Rates of the composts were balanced with amounts of added nitroge, such as 6.0 g N per pot. A one-factor experiment was conducted in Kick-Brauckmann pots, kept in a greenhouse at the University of Warmia and Mazury in Olsztyn. After harvest, fresh matter of stems and leaves as well as corn cobs was determined. Next, the plant samples were dried to determine the dry matter content and finally, after mineralisation, the concentration of Cu, Zn and Mn was determined by atomic absorption spectrophotometry. The composts significantly reduced the concentration of zinc and manganese in the vegetative yield of cor (lstems and leaves). Urea applied in combination with the composts very strongly increased the concentration of manganese and, to a lesser degree, the level of zinc and copper in vegetative organs. In corn cobs, the application of urea increased only the content of manganese. Urea had a stronger effect on increasing the weight of cobs rather than the vegetative mass of corn plants. The uptake of Cu, Zn and Mn was more evidently conditioned by the accumulation of these elements in dry matter than by the total weight of corn plants. The extent of the relationship between the uptake of Cu, Zn and Mn and their concentration of corn biomass is expressed the by corresponding correlation coefficients: 0.66, 0.65 and 0.68.

Key words: corn, composts, urea, Cu, Zn and Mn.

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DZIAŁANIE MOCZNIKA STOSOWANEGO ŁĄCZNIE Z KOMPOSTAMI NA ZAWARTOŚĆ Cu, Zn I Mn W ZIELONEJ MASIE KUKURYDZY

Abstrakt

W doświadczeniu wazonowym uprawiano kukurydzę na zieloną masę, którą nawożono kompostami lub kompostami z dodatkiem 3 g N na wazon (mocznik). Komposty założono w skrzyniach z desek o wymiarach 50×60×60 cm. Okres kompostowania wynosił 3,5 miesiąca, do ustabilizowania temperatury równej otoczeniu. Dawki kompostów były zrównoważone ilością wprowadzonego azotu, tj. 6,0 g N na wazon. Doświadczenie jednoczynnikowe przeprowadzono w hali wegetacyjnej UWM w Olsztynie, w wazonach typu Kick-Brauckmanna. Po zbiorze kukurydzy określono świeżą masę łodyg i liści oraz kolb, po wysuszeniu – zawartość suchej masy, po mineralizacji próbek oznaczono koncentrację Cu, Zn i Mn metodą absorpcyjnej spektrometrii atomowej. Komposty istotnie ograniczały koncentrację cynku i manganu w wegetatywnym plonie kukurydzy (łodygi + liście). Mocznik zastosowany łącznie z kompostami bardzo silnie zwiększał koncentrację manganu oraz w mniejszym stopniu cynku i miedzi w organach wegetatywnych. Pod wpływem mocznika w kolbach zwiększyła się jedynie zawartość manganu. Mocznik silniej zwiększał masę kolb niż masę wegetatywną kukurydzy. O pobraniu Cu, Zn i Mn w większym stopniu decydowało ich nagromadzenie w suchej masie niż całkowita masa kukurydzy. Siłę zależności pobrania Cu, Zn i Mn od ich zawartości w biomasie kukurydzy określają współczynniki korelacji, odpowiednio: 0,66, 0,65 i 0,68.

Słowa kluczowe: kukurydza, komposty, mocznik, Cu, Zn, Mn.

INTRODUCTION

Corn is one of the most popular crops. Discovered in Central America, it was brought to Europe in 1494, after the second travel of Christopher Columbus to America. At that time it was described as a very large plant with a beautiful stem and golden grain. Corn is a popular crop mainly because it is capable of producing high yields, consisting of nutritious green matter and grain (Bilski et al. 1997). However, for the plant to give large, good quality yields, it needs to be supplied with suitable nutrients. Corn is very sensitive to organic and mineral fertilization (Sienkiewicz 2003).

Apart from macronutrients, corn often needs microelements (Spiak 2000). Sound fertilization regimes can satisfy plants' requirements for micronutrients (Mazur, Mazur 2002, Rutkowska et al. 2002). Plants which receive all necessary nutrients are able to produce large yields which are of superior nutritional quality. This also concerns micronutrients, which are better to be given to animals in feeds and to people in foodstuff than as chemical supplements.

Using bio-waste, such as sludge, in agriculture is still a controversial issue. Processed (composted) sewage sludge generates much better results than fresh sludge (Hermann, Harasimowicz-Hermann 2006). Concern is raised by the trace elements which can enter soil along with sewage sludge

(McBride et al. 2004, Bowszys et al. 2007). This can lead to excessive accumulation of such elements in plants (Rattan et al. 2005), especially when grown on moist soils (Diatta 2008).

The purpose of this study has been to analyse the influence of urea applied in combination with various composts on the content of three micronutrients (Cu, Zn and Mn0 in corn.

MATERIAL AND METHODS

Sewage sludge from the wastewater treatment plant in Olecko was used to produce composts. It was composted with sawdust, lignite and molasses brewing extract (Table 1). The composts were placed into wooden boxes measuring 50x60x60 cm located at the Wastewater Treatment Plant in Olecko. They were composted for 3.5 months, that is until the temperature inside the composts became stable and equal the ambient temperature. The composts were preserved for the pot trials. Kick-Brauckmann pots were filled with 9 kg of soil taken from the arable horizon and previously mixed with the composts. The grain size distribution of the soil material was as follows: 44% sand, 39% dust and 7% floatable particles. The content of organic carbon and total nitrogen was 10.9 and 0.54 $g \cdot kg^{-1}$, respectively. The soil was moderately abundant in K and Mg but low in P whereas the concentration of available micronutrients (Cu, Zn and Mn) was within the middle range.

Table 1

Content of macro- and micronutrients in composts

Compost	Composition^{x}	N	P	K	Mg	Cu	Zn	Mn
		$g \cdot kg^{-1}$ of dry matter				$mg \cdot kg^{-1}$ of dry matter		
1	70% sludge + 30% sawdust	20.20	9.55	7.69	1.54	106.2	210.7	191.5
2	70% sludge + 20% sawdust + 10% lignite	21.90	12.19	9.40	2.71	118.3	221.3	209.9
3	60% sludge + 20% sawdust +10% lignite + 10% extract^{xx}	21.00	18.24	14.51	2.65	98.5	205.9	180.6

^{x}dry matter, ^{xx}fresh matter

A one-factor experiment with four replications was conducted in a greenhouse, at the UWM in Olsztyn. After emergence, 7 plants were left in each plot. The corn plants were harvested after 80 days of vegetative growth.

The experiment consisted of 7 objects:

1) no fertilization (control),
2) compost 1,
3) compost 2,
4) compost 3,
5) compost 1 + urea (3 g N per pot),
6) compost 2 + urea (3 g N per pot),
7) compost 3 + urea (3 g N per pot).

The rates of the composts applied were balanced with the amounts of nitrogen introduced to the post, such as 6.0 g N per pot. After harvest, fresh yield was determined (stems + leaves, and corn cobs). Once the samples were dried and mineralized, the concentration of Cu, Zn and Mn was determined with the AAS method. The results underwent statistical processing, using analysis of variance for a one-factor experiment.

RESULTS

The fertilization treatments differentiated the content of copper, zinc and manganese in dry matter of stems and leaves as well as cobs (Table 2). The composts led to a considerable reduction in the concentration of zinc and manganese in the vegetative mass of corn (stems + leaves). This may have been caused by the enhanced binding of these metals to the organic matter introduced to soil along with the composts. Organic matter strongly inhibits the uptake of trace elements by plants (SAHA et al. 1999). Weber et al. (2003) claims that composts improve the base saturation of the sorptive complex, which limits the uptake of trace metals. No such dependence occurred for copper, although this metal is very strongly bounded to organic

Table 2

Content of Cu, Zn and Mn in corn (mg·kg^{-1} of dry mattrer)

Fertilization	Cu		Zn		Mn	
	leaves and stems	corncobs	leaves and stems	corn cobs	leaves and stems	corn cobs
Control	4.96	-	35.56	-	62.12	-
Compost 1	5.09	7.60	11.82	71.64	36.20	29.24
Compost 2	4.64	7.13	24.44	47.38	36.88	21.96
Compost 3	5.41	8.19	24.88	52.76	41.48	18.90
Compost 1 + urea	6.63	7.75	35.74	54.00	130.14	35.52
Compost 2 + urea	5.96	5.20	39.98	51.80	134.50	27.72
Compost 3 + urea	4.96	6.00	22.94	44.92	107.90	22.62
$LSD_{0.01}$	0.60	067	0.37	0.97	0.54	0.33

matter. It can be suspected that during the mineralization process, more available copper was present in the soil. In turn, urea applied along with one of the composts very strongly increased the concentration of manganese and, to a lesser degree, the levels of zinc and copper in the vegetative parts of corn plants. Under the effect of urea, corn cobs were found to be richer only in manganese. The increase in the concentration of these metals in corn is attributable to their greater availability in soil, which was improved by soil's acidification due to nitrification of ammonium (following the ammonification of urea). It can also by hypothesized that translocation of Cu and Zn to cobs was restricted to a greater extent than that of manganese. Comparison of the means from the series with the composts and the objects receiving the composts with urea showed that N-min significantly differentiated the content of the micronutrients. Assuming that the content of the micronutrients in the series without N0min was 1.0, any increase or decrease in the concentration of the elements is represented by the following values:

Micronutrient	leaves and stems	cobs
Cu	1.16	0.83
Zn	1.61	0.88
Mn	3.18	1.19

The above proves that the N-min fertilization had the strongest influence on the content of Mn, while affecting the most weakly the level of Cu. The amounts of the trace elements in corn are different from the ones reported by SĄDEJ et al. (2004). Considering the mean values, the Cu concentration was approximately 2-fold higher in our study than in the cited paper. The difference in the content of Mn was even greater. Kaczor et al. (2006), who tested oilseed rape fertilized with sewage sludge, obtained very high concentration of Mn in plants during the inflorescence stage and in straw and seeds after harvest. In turn, the concentrations of zinc reported by SĄDEJ et al. (2004) was only slightly higher than that obtained in our study. According to WEBER et al. (2002), a single dose of sludge, be it a high one, containing large amounts of trace elements may not lead to soil pollution or raised levels of such elements in plants, even when they grow on light soils. DE HANN (1981) claims that higher availability of nitrogen in environment favours increased concentrations of trace elements in plants. The present tests involved urea, which significantly raised the concentration of Cu, Zn and Mn in corn.

Also the quantities of fresh and dry matter of corn per pot were significantly varied depending on the fertilization system (Table 3). Relative to the control, fertilization with the composts alone caused a three-fold increase in green matter. A combination of a compost and urea led to a further increase of fresh weight, now corn yielded 3.2-fold more green matter than the control object. Plants from the two objects without N-min. had more dry matter. This was reflected in the dry matter yield, which was 3.4 and 3.5-fold

Table 3

Fresh and dry master of maize in g per pot

Fertilization	Fresh matter			Dry matter		
	leaves and stems	corn cobs	sum	leaves and stems	corn cobs	sum
Control	239.0	-	-	68.8	-	-
Compost 1 Compost 2 Compost 3	706.7 696.0 676.0	36.0 48.3 41.6	742.7 744.3 717.6	259.7 246.4 212.3	3.5 5.7 5.2	257.2 252.1 217.5
Compost 1 + urea Compost 2 + urea Compost 3 + urea	876.7 928.0 874.7	415.0 401.6 460.0	1291.7 1329.6 1334.7	225.3 246.8 255.4	56.8 52.2 68.5	282.1 299.0 323.9
$LSD_{0.01}$	114.3	90.1	123.1	240.0	11.9	39.5

higher, respectively, than the control. Mineral nitrogen produced a particularly strong influence on the weight of cobs, whose fresh matter increased by 101.3% and dry matter by 123.35 versus the corn growing on soil fertilized exclusively with the composts.

The uptake of the analyzed micronutrients by corn harvested for green matter depended on fertilization (Table 4). Compared to the control, higher uptake was observed in the objects fertilized with one of the composts together with N-min. than receiving only a dose of compost. The improved uptake of micronutrients stimulated by N-min. was: 44.7% for Cu, 116.1% for Zn and 232.4% for Mn. With such a large increment in the assimilation of manganese, the relative ratio of Cu and Zn in the total mass of micronutrients taken up by plants was lower than in the series which did not receive N-min.

Table 4

Uptake of microelements (g per pot)

Fertilization	Cu	Zn	Mn
Control	0.34	2.45	4.27
Compost 1 Compost 2 Compost 3	1.32 1.18 1.19	3.25 6.29 5.55	9.18 9.09 8.81
Mean	1.23	5.03	9.03
Compost 1 + urea Compost 2 + urea Compost 3 + urea	1.93 1.74 1.68	11.11 12.57 8.94	29.32 33.19 27.56
Mean	1.78	10.87	30.02

The calculated correlations have demonstrated that the uptake of Cu, Zn and Mn was more strongly shaped by the accumulation of these elements in dry matter (weighted average of the content of micronutrients in vegetative parts: stems + leaves, and in cobs) than the total yield of corn. The dependence between the uptake of Cu, Zn or Mn and the content of these elements corn biomass is defined by the values of correlations: 0.66, 0.65 and 0.68, respectively. The lower values of the correlation coefficients obtained for the relationship between the uptake of the metals and corn yield suggest that copper, zinc and manganese could not be implied as factors limiting the growth of corn, neither when they are insufficient nor when they appear in excess in soil.

CONCLUSIONS

1. Urea used in combination with composts very strongly increased the concentration of manganese, less so the content of zinc and copper in vegetative parts of corn (stems and leaves). In corn cobs, it was only the level of manganese that increased under the influence of urea.

2. The uptake of Cu, Zn and Mn by corn is conditioned by the accumulation of these metals in dry matter rather than the total dry matter yield.

3. Sewage sludge-based composts with various added materials (sawdust, lignite or extract) can be used to fertilize corn.

4. Urea applied along with composts can have a favourable influence on the quality of green matter used for production of corn silage as such fertilization leads to increased contribution of cob weight to the total vegetative mass of harvested corn.

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CONTENT OF POTASSIUM AND MAGNESIUM IN ORGANIC SOILS AND MEADOW VEGETATION OF SZCZECIN POMERANIA

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Abstract

The studies included the major organic meadow soils of Szczecin Pomerania, left fallow or sporadically used extensively. The following determinations were made: the content of plant available magnesium and potassium (using HCl at the concentration of 0.5 mol·dm^{-3}), their total forms (soluble in the mixture of concentrated acids HNO_3 + $HClO_4$) as well as the content of potassium and magnesium in the meadow-pasture sward from the area under study. The results are presented in Table 1. The investigated peat-muck, gyttia-muck, mineral-muck and muckous soils, in the surface layer 0-30 cm deep (which was primarily the muck layer) mostly contained the amounts of potassium and magnesium typical of organic soils when soluble in the mixture of concentrated acids HNO_3+$HClO_4$ but low and frequently very low amounts of potassium soluble in 0.5 mol·dm^{-3} HCl from (0.04 to 0.51g·kg^{-1}). The content of this form of potassium depended on the degree of peat siltation. Low resources of available potassium were caused by the deficiency of this element in the meadow pasture sward since only in the sward of the Gryfinski Polder in Miedzyodrze and the sward from gyttia-muck soils near Miedwie Lake the optimum amounts were detected (above 15.0 g·kg^{-1} dry matter). In comparison with these results, the content of magnesium, soluble in 0.5 mol·dm^{-3} HCl in these soils was more favourable to plants (generally above 0.40 g·kg^{-1}), which is considered high according to the Institute of Soil Science and Plant Cultivation (IUNG 1990). In meadow sward, magnesium content mostly exceeded 2.0 g·kg $^{-1}$ dry matter so either approached or reached the optimum value for fodder. Despite this, the calculated K:Mg ionic ratios confirm an unfavourable fodder value.

Key words: organic soils, soil content of potassium and magnesium, sward content of potassium and magnesium.

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ZAWARTOŚĆ POTASU I MAGNEZU W GLEBACH ORGANICZNYCH I ROŚLINNOŚCI ŁĄKOWEJ POMORZA SZCZECIŃSKIEGO

Abstrakt

Badaniami objęto ważniejsze obszary organicznych gleb łąkowych Pomorza Szczecińskiego, które znajdują się w stanie odłogowania lub są tylko sporadycznie ekstensywnie użytkowane. Określono w nich zawartość dostępnego roślinom magnezu i potasu (stosując HCl o stężeniu 0,5 mol•dm^{-3}) oraz formy ogólne (rozpuszczalne w mieszaninie stężonych kwasów HNO_3+$HClO_4$) tych pierwiastków. Ustalono także zawartość potasu i magnezu w runi łąkowo-pastwiskowej porastającej badany teren.

Badane gleby torfowo-murszowe, mułowo-murszowe, gytiowo-murszowe, mineralno-murszowe i murszaste w powierzchniowej 0-30 cm warstwie (stanowiącej przeważnie poziom murszowy tych gleb) na ogół zawierały typowe dla gleb organicznych wartości potasu i magnezu rozpuszczalnego w mieszaninie stężonych kwasów HNO_3+$HClO_4$. Wykazywały natomiast niskie, a często nawet bardzo niskie ilości potasu rozpuszczalnego w HCl o stężeniu 0,5 mol·dm^{-3} (od 0,04 do 0,51 g·kg^{-1}). Przy czym na zawartość tej formy potasu duży wpływ wywierał stopień zamulenia torfu. Stan niskiej zasobności gleb w dostępny roślinom potas wywołał niedobór tego pierwiastka w runi łąkowo-pastwiskowej, bowiem tylko w runi Polderu Gryfińskiego w obrębie Międzyodrza oraz ruń na glebach gytiowo-murszowych w pobliżu jeziora Miedwie wykazano jego optymalną ilość (powyżej 15,0 g·kg^{-1}s.m.). Na tym tle badane gleby odznaczały się korzystną dla roślin zawartością magnezu rozpuszczalnego w HCl o stężeniu 0,5 mol·dm^{-3} (z reguły powyżej 0,40 g·kg^{-1}), co jest zasobnością wysoką. W runi łąkowej zawartość magnezu na ogół przekroczyła 2,0 g·kg^{-1} s.m., czyli zbliżyła się lub osiągała wartość optymalną dla pasz. Pomimo to wyliczone proporcje jonowe K:Mg potwierdzają niekorzystną wartość paszy.

Słowa kluczowe: gleby organiczne, potas i magnez w glebach, potas i magnez w runi łąkowej.

INTRODUCTION

According to the Central Statistical Office, in 2007 permanent meadows and pastures covered about 3.3 mln ha in Poland, nearly 10.5% of the country's total area. However, in recent years the pastures have been shrinking. In comparison with 1980, 3.2-fold less land is under pastures now, which may be attributed to the reduction in cattle stock from 12.6 mln in 1980 to 5.6 mln in 2006. Apparently, this must have happened over the area formerly occupied by state farms, including the region of Szczecin Pomerania, where permanent meadows and pastures make up 14% of agricultural acreage.

In Szczecin Pomerania, permanent grassland (over 80% of total area) occurs predominantly on organic soils (peat-muck, mud-muck, gyttia-muck, mineral-muck and muckous soils). They used to be the source of fodder for cattle breeding on state farms. As a result of intensive grassland management, especially NPK fertilization, up to 3 hay cuts were harvested. As early as 1990 a reverse trend appeared and many meadows and pastures of Szczecin Pomerania were left fallow.

The present study focuses on chemical properties of fallowed or sporadically extensively used soils under permanent grassland and chemical content of their vegetation cover.

This paper is the synthesis of study results on the content of magnesium and potassium in the above elements of natural habitat.

MATERIAL AND METHODS

Since 1995, the Department of Soil Science of Szczecin Agricultural University has been investigating chemical properties of organic soils under permanent grassland. So far the studies have included the largest meadow complexes of Szczecin Pomerania which have been left idle or sporadically extensively grazed with cattle or horses. The studies were conducted in the Ina valley (Wapnica-Suchanówko), the Odra valley (Marwice-Gryfino), Międzyodrze (mainly in its southern part with the Widuchowski Polder, southern part of the Gryfiński Polder and Pucka Isle), Miedwie Lake and the meadows along the Baltic Sea coast with halophytic vegetation (objects: the Dziwna valley near Jarzębowo, Chrząszczewska Isle, the Rega valley near Włodarka). The study objects are presented in Figure 1. Usually, both the topsoil and the peat thickness were examined. Over 1000 soil samples and 260 samples of pasture vegetation were analysed. The sward samples were collected at the end of June and the beginning of July, and mineralised in a 1:1mixture of concentrated HNO_3 + $HClO_4$.

In order to determine the content of available forms of magnesium and potassium, a solution of HCl at the concentration 0.5 $mol \cdot dm^{-3}$ was used according to the recommendation of the Institute of Soil Science and Plant Cultivation in Puławy (IUNG, 1990). Approximate total values of these elements were obtained by soil mineralisation in a mixture of concentrated acids HNO_3+$HCIO_4$. Potassium forms were determined using flame spectrophotometry and magnesium content – by atomic absorption spectrophotometry.

General characterisation of study area

This research was conducted on organic meadow soils of Szczecin Pomerania developed from lowmoor peat of varying siltation, especially in the upper part of soil profile, classified as peat-muck soils according to the Classification of Polish Soils (1989), Fibric Histosols according to WRB classification 1998 and, near Miedwie Lake, shallow organic-carbonate soils on lacustrine chalk, included in gyttia-muck soils (according to WRB 1998 – Saprihistic Gleysols).

KOCHANOWSKA and RYGIELSKI (1994) point out that these soils constitute vast meadow complexes, measuring 200-4,000 ha, and that 40% of their area

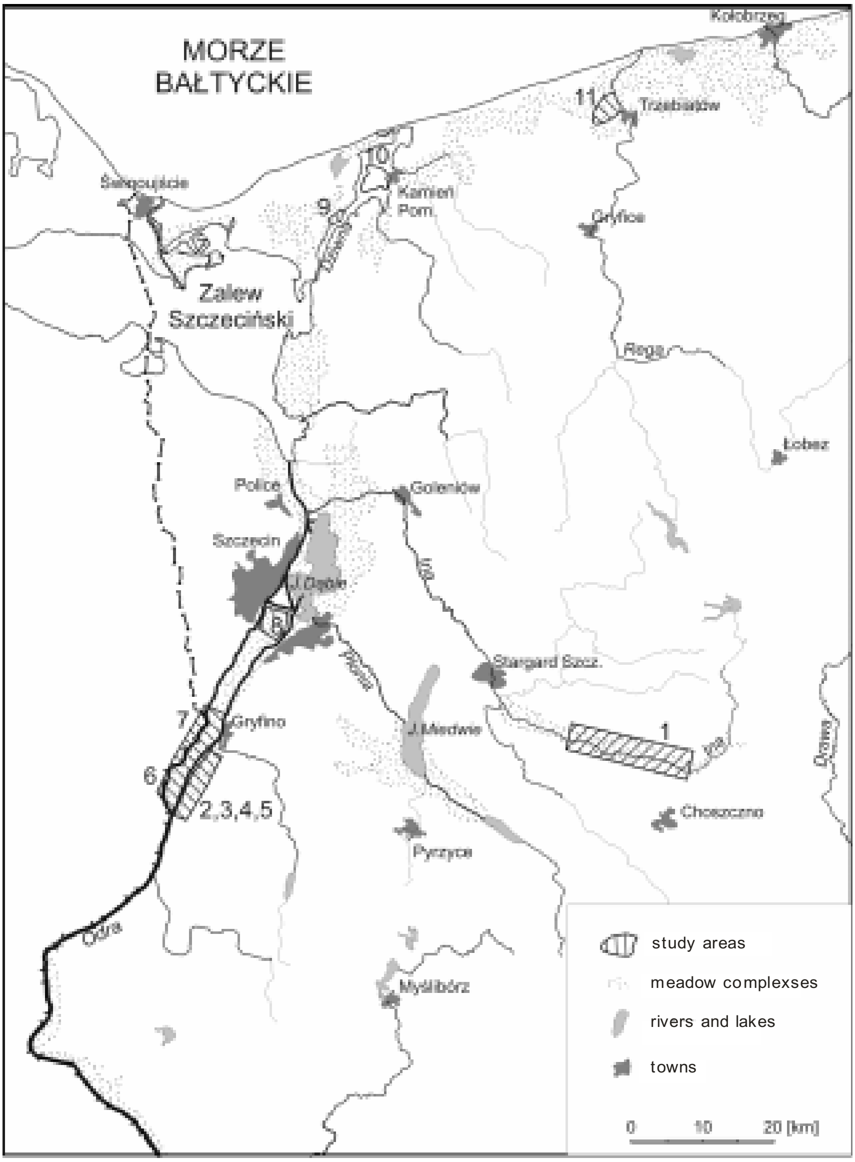

Fig. 1. Distribution of studied areas (1-10: study objects according to Table 1)

exists due to the polder drainage system. Siltation processes are typical of valley organic soils but in the case of the Odra valley and the Miedzyodrze region, a great role in their intensification is played by river regulation works and a parallel construction of the waterway Świnoujście-Szczecin. These works provided considerable amounts of material from the deepened water bodies, which were spread on the adjacent lower areas to enhance their fertility (BOROWIEC, KWARTA 1959).

In the years 1960-1980 the valley meadows in the Province of Szczecin were drained (occasionally by pumping stations) and reclaimed (frequently using all cultivation practices), which intensified the development of animal farms and dry goods production. That period has been documented in many publications, for example RYGIELSKI (1992). During the intensive management of meadows and pastures owned by state farms, doses of mineral fertilisation were as follows: N – above 180, K_2O – 80-140, P_2O_5 – 40-80 $kg \cdot ha^{-1}$, plus sporadic applications of magnesium .

As a result of the economic transformations of the 1990s, most of these meadows and pastures ceased to be utilized and fertilized, which led to the abundant appearance of common reed rushes, scrub and then woodland. Only the EU subsidies for agriculturally used land encouraged grassland owners to cut them once or utilize as pasture or arable land.

This paper presents the content of potassium and magnesium only in the surface layer (0-30 cm), being the muck horizon or its upper part, and its vegetation cover. Muck horizon develops from peat transformation in natural processes occurring during drainage of organic soils. These processes bring about the gradual disappearance of primarily fibrous or spongy peat structure in the direction of tiny aggregates contributing to humus formation and immobilization of essential plant nutrients.

According to OKRUSZKO and SAPEK (1991), the muck formation process results in the increase in the total content of chemical elements such as potassium, phosphorus and iron. Therefore, the level of muck formation affects the development of grass communities and soil agricultural value. Transformations in the soils developed from lowmoor peat, taking into consideration biological and chemical changes, coupled with the changes in the soil composition, with the emphasis on the role of mud formation process have been discussed in detail by OKRUSZKO (1993) and OKRUSZKO and ILNICKI (2003).

After meadow soils had ceased to be utilized, their topsoil (0-30 cm) was found to have a high organic matter content (20- 85%) and varying reaction (pH_{KCl} 4.1-6.5). The only exception was the alkaline soil (pH_{KCl} 7.3-7.8) in the carbonate-muck horizon.

RESULTS AND DISCUSSION

In the surface layer (0-30 cm), mostly muck or mud-muck, the mean values for the content of potassium soluble in 0.5 $mol \cdot dm^{-3}$ HCl varied from extremely low to low according to the IUNG standards (1990) and SAPEK and SAPEK (1997). In was only in the Odra valley that this value exceeded

0.50 g·kg^{-1} dry matter, which indicates medium availability. The analysis of variations in the content of this element proves that the resources of available potassium in the examined soils are unfavourable. The highest content (0.19-0.51 g·kg^{-1} dry matter on average) was observed in the strongly silted muck layer, which occurs predominantly in the valley of the Lower Odra, especially in Międzyodrze and at Marwice, where the highest content of potassium soluble in the mixture of concentrated HNO_3+$HCIO_4$ (Table 1) was also found, ranging on average from 0.60 to 4.71 g·kg^{-1} dry matter. The smallest amounts were obtained from the degraded, strongly dried out soils in the vicinity of the power plant Dolna Odra (mean 0.60 g·kg^{-1} dry matter). The content of this form of potassium drastically fell below the 0-30 cm layer with weakened siltation in lowmoor peat (NIEDZWIECKI 2000). Such regularity did not occur in the silted peat deposit in Międzyodrze. In the mud or gyttia-muck layers (organic-carbonate soil on lacustrine chalk; MELLER 2006) the content of potassium soluble in the mixture of HNO_3+ + $HClO_4$ was within the range 0.33-0.55 g·kg^{-1} dry matter (mean 0.46 g·kg^{-1}) and was decreasing with depth to 0.11-0.25 g·kg^{-1} dry matter (mean 0.16 g g·kg^{-1} dry matter). Before World War II attention had been paid during the Odra regulation works to the need of fertilizing meadow soils with potassium, as it can be seen from WAGNER'S papers (1921), suggesting that hay of good nutritive value should contain 1.7% of potassium, and the data presented by HONCZARENKO (1961) concerning pre-war grassland fertilization per 1ha in Szczecin region: pure N to 36.7 kg, P_2O_5 29-32 kg and K_2O 38-50 kg. On the basis of German and his own studies, conducted in the years 1955-1961 he stated that there is high profitability of potassium fertilization on peat soils of Szczecin province. NIEDZWIIECKI (2000) and NIEDZWIECKI et. al. (2006) also noticed low resources of available potassium in organic soils of Szczecin Pomerania. Similar evaluation of organic soils in other lowland regions of Poland is given by PIAŚCIK and ŁACHACZ (2001) and BRANDYK et.al. (2001), who described the content of potassium as extremely poor.

Low resources of available potassium in organic soils result from the discontinuation of K fertilization, which is confirmed by OKRUSZKO and SAPEK (1991) and SIGUA et. al. (2006) in their studies on marshlands with a high organic matter content. These authors emphasize that peat soils with a low content of mineral clay have low potassium holding capacity due to weak bonds in the sorption complex and easy potassium ion dislodgement by other cations, mainly calcium and magnesium.

At the same time, there was hardly any interest in magnesium content in these soils as the cited literature shows, probably due to the belief that NPK fertilization covers all the needs of grassland vegetation.

The values of magnesium, soluble in HCl at the concentration 0.5 mol·dm^{-3} (available forms), are favourable since, as a rule, they exceed 0.40 g·kg^{-1}, which means that according to IUNG (1990) there is either high or very high content of this element in the examined soils. The mead-

Table 1

Content of potassium and magnesium in surface layer (0-30 cm) of organic, peat-muck, peat-mud-muck soils and their vegetation cover ($g \cdot kg^{-1}$)

	Study object	K soluble in 0.5 mol·dm^{-3} HCl		K in concentrated HNO_3+$HClO_4$		Mg soluble in 0.5 mol·dm^{-3} HCl		Mg in concentrated HNO_3+$HClO_4$		Mean content in sward dry matter		
		min-max	$\bar{x}$	min-max	$\bar{x}$	min-max	$\bar{x}$	min-max	$\bar{x}$	K	Mg	K:Mg
1	Ina valley	0.04-0.07	0.05	1.16-1.51	1.33	0.29-0.53	0.43	1.92-2.06	1.99	4.0	2.7	0.46
2	Odra valley (at Dolna Odra Power Plant) – dried area	0.06-0.34	0.16	0.18-1.75	0.60	0.24-1.33	0.64	0.34-4.36	1.46	7.8	4.3	0.56
3	Odra valley near ash dumping ground	0.08-0.42	0.19	0.63-6.17	1.91	0.36-1.41	0.78	1.39-5.38	2.83	12.6	3.4	1.14
4	Odra valley – Krajnik	0.04-0.23	0.13	0.38-1.84	1.41	0.67-0.90	0.80	1.26-2.84	2.32	6.5	3.8	0.53
5	Odra valley – Marwice	0.41-0.56	0.51	3.57-5.37	4.71	0.88-1.14	0.99	2.33-2.92	2.65	13.8	2.2	1.94
6	Międzyodrze – Widuchowski Polder	0.09-0.42	0.20	1.99-4.75	3.09	0.81-1.08	0.92	1.99-5.35	3.53	7.3	2.6	0.87
7	Międzyodrze – Gryfiński Polder	0.08-1.05	0.38	0.82-6.50	3.66	0.72-1.15	0.92	1.56-6.70	3.99	17.9	1.6	3.46
8	Międzyodrze – Pucka island	0.01-0.31	0.19	0.25-2.08	1.38	0.44-1.01	0.67	0.62-3.08	1.78	9.3	4.7	0.61
9	Pre-Baltic area – Dziwna valley (Jarzębowo)	0.05-0.38	0.19	1.39-5.10	2.91	0.45-2.30	1.24	1.84-5.24	3.45	15.3	1.3	3.64
10	Pre-Baltic area – Chrząszczewska island	0.02-0.06	0.04	0.74-5.01	2.90	0.41-0.61	0.57	0.96-5.35	3.30	12.3	2.8	1.36
11	Rega valley – Włodarka	0.04-0.15	0.08	0.92-3.16	1.92	0.31-0.65	0.57	1.73-3.13	2.36	12.0	2.3	1.61

ow soils in the valley of the Dziwna (Jarzębowo), the Odra, and Międzyodrze, appeared to be the richest in the forms of available magnesium (over 0.80 $g \cdot kg^{-1}$). The content of magnesium soluble in the mixture of concentrated acids was several times higher (Table 1). The amounts of both forms of magnesium were growing with the growing thickness of peat deposit. In the gyttia-muck soils near Miedwie Lake, in the muck layer, the total amount of this element ranged from 2.30 to 6.96 $g \cdot kg^{-1}$ dry matter (mean 4.26 $g \cdot kg^{-1}$ dry matter) and in calcareous gyttia (at the depth 30-50 cm) increased to 7.05 $g \cdot kg^{-1}$ dry matter on average. Nutrient uptake by sward depends on many factors such as the kind and level of fertilization (FALKOWSKI et.al. 2000, GRABOWSKI et.al. 2006), floristic composition of meadow and pasture communities. However, in agricultural practice the mineral composition of grassland fodder from sward is given without taking into account its floristic composition. In the opinion of OSWIT and SAPEK (1982) the content of potassium and magnesium in the grasses of Polish meadows is as follows: K – 14.1, Mg – 1.6 $g \cdot kg^{-1}$ dry matter. Good quality fodder according to the recommendations of CZUBA and MURZYŃSKI (1989) FALKOWSKI et. al. (2000) should contain 17-20 g K and 3.0g $Mg \cdot kg^{-1}$ dry matter. Minimum content of potassium, essential to animals, should amount to 10 $g \cdot kg^{-1}$ dry matter, and magnesium – 2.0 $g \cdot kg^{-1}$ dry matter. In NOWAK's opinion (1983) maximum potassium content should not exceed 20.0-25.0 $g \cdot kg^{-1}$.

Mean values of potassium content reveal the deficiency of this element since only the sward from the Gryfiński Polder, in Międzyodrze, contained the optimum amount, which was reported by NIEDŹWIECKI et. al. (2008), whereas the minimum amounts (minimum indispensable for fodder) were recorded in 5 study objects and in the remaining ones they were assessed as low. A relatively favourable potassium content (15.0-17.5 $g \cdot kg^{-1}$) was found in the analysed vegetation cover from gyttia-muck soils.

Compared to these results, the magnesium content in meadows and pastures under study is much better, with the dominant values above 2.0 $g \cdot kg^{-1}$ dry matter (Table 1) and only two objects scoring below that value. It should be noted that the magnesium deficiency in sward is accompanied by the greatest potassium accumulation. Content of magnesium in pasture grass from gyttia-muck soils ranged from 1.5 to 3.1 g kg dry matter.

Our results concerning the content of potassium and magnesium are to a great extent consistent with NOWAK'S studies (1983), in which he determined hay mineral resources during grassland intensive fertilization. His data show that in the vicinity of Szczecin only a few hay samples contained excessive amounts of potassium whereas hay from Szczecin Province had a more advantageous content of magnesium in comparison with low magnesium resources in the whole country

The calculated K:Mg ionic ratios confirm unfavourable value of fodder from the examined grassland objects. The optimum K:Mg ratio in fodder equals 6:1 and should not be lower than 2-6:1.

CONCLUSIONS

1. Fallow or extensively used organic soils of Szczecin Pomerania were generally found to have a low content of available potassium and medium to high magnesium content in their 0-30 cm layer.

2. Grassland sward from the investigated objects was deficient in potassium but had a more favourable amount of magnesium. Calculated ionic proportions of K:Mg confirm its unfavourable fodder value.

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POLYPHENOLIC COMPOUNDS AND BIOELEMENTS IN FRUITS OF EASTERN TEABERRY (*GAULTHERIA PROCUMBENS* L.) HARVESTED IN DIFFERENT FRUIT MATURITY PHASES

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Abstract

Eastern teaberry (*Gaultheria procumbens* L.) is known for its high content of essential oils in leaves and fruits, which are used in the pharmaceutical, food and cosmetics industries. Recently, teaberries have been attracting more interest owing to their content of polyphenolic compounds. The purpose of our study has been to determine the content of polyphenolic compounds (anthocyanins and total phenols) as well as their antioxidative activity and the concentration of several bioelements (Ca, Mg, Fe and Zn) in extracts from fruits of Eastern teaberry harvested in three different fruit maturity stages. The content of polyphenolic compounds depended on the harvest date. The highest level of these compounds was found in extracts from teaberries collected in the full maturity phase. All teaberry extracts, independently of the harvest date, demonstrated high antioxidative activity. Among the bioelements determined, teaberry extracts contained more calcium than magnesium and more iron than zinc. The content of such bioelements as Mg, Fe and Zn (in contrast to Ca) found in teaberry extracts did not depend on the harvest date.

Key words: anthocyanins, antioxidative activity, bioelements, fruits, *Gaultheria procumbens*, phenols.

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ZWIĄZKI POLIFENOLOWE I BIOPIERWIASTKI W OWOCACH GOLTERII ROZESŁANEJ (*GAULTHERIA PROCUMBENS* L.) ZBIERANEJ W RÓŻNYCH TERMINACH DOJRZAŁOŚCI

Abstrakt

Golteria rozesłana jest rośliną znaną z zawartości w owocach i liściach olejków eterycznych, które są wykorzystywane w przemyśle farmaceutycznym, spożywczym i perfumeryjnym. W ostatnich latach owoce golterii rozesłanej wzbudzają też zainteresowanie ze względu na zawartość związków polifenolowych. Celem pracy było oznaczenie zawartości związków polifenolowych (antocyjanów i fenoli ogółem) i ich właściwości antyoksydacyjnych oraz zawartości biopierwiastków (Ca, Mg, Fe, Zn) w ekstraktach z owoców golterii rozesłanej zbieranej w trzech terminach dojrzałości. Zawartość związków polifenolowych w ekstraktach z owoców golterii rozesłanej zależała od terminu zbioru owoców. Najwięcej związków polifenolowych zawierały ekstrakty z owoców zbieranych w okresie pełnej dojrzałości. Niezależnie od terminu zbioru, ekstrakty z owoców golterii rozesłanej wykazywały bardzo dużą zdolność antyoksydacyjną. Spośród badanych biopierwiastków, ekstrakty z owoców golterii rozesłanej zawierały więcej wapnia niż magnezu oraz więcej żelaza niż cynku. Zawartość takich biopierwiastków, jak Mg, Fe, Zn (wyjątek Ca), w ekstraktach z owoców golterii rozesłanej nie zależała od terminu zbioru owoców.

Słowa kluczowe: antocyjany, aktywność antyoksydacyjna, biopierwiastki, fenole, golteria rozesłana, owoce.

INTRODUCTION

Ornamental cover plants, increasingly more common in gardens, parks and greens, have been drawing more and more attention in the recent years (ALEKSANDROVA 1981, MAROSZ et al. 2000, WAŻBIŃSKA 2000, ZARAŚ et al. 2000). Eastern teaberry, also known as checkerberry, boxberry or American wintergreen (*Gaultheira procumbens* L.), is one of such plants. It is low, evergreen shrub with creeping shoots. In July and August it is in bloom, producing white or pink bell-shaped flowers (FRAZIK 1991). Round, glistening pink, bag-shaped fruits, 8-15 mm in diameter, remain on the plant until spring the following year (CZEKALSKI 2006). Fruits and leaves of Eastern Teaberry contain essential oils, which have a medicinal use in treatment of skin and throat inflammation. In the United States, these oils are used in the food (to make chewing gums and sweets), cosmetics and pharmaceutical industries. This plant is very popular in Central and South Americas, where it is known as teaberry and used to make aromatic infusion (CLARC 1999, HUFFMAN et al. 1994, RIBNICKY et al. 2003, SENETA, DOLATOWSKI 1997). Fruits of Eastern teaberry, owing to their content of polyphenols (catechins, quercetins), have antixodative properties (ACUNA et al. 2002, MA et al. 2001).

The principal aim of the research has been to determine the content of polyphenolic compounds (anthocyanins and total phenols), their antioxidative activity and the concentration of bioelements (Ca, Mg, Fe and Zn) in extracts from Eastern teaberry fruits harvested on three different dates. In addition, correlation between the total phenolic content and the level of anthocyanins as well as the correlation between the content of polyphenols and antioxidative activity in extracts from Eastern teaberry fruits were established.

MATERIAL AND METHODS

The material consisted of fruits of Eastern teaberry (*Gaultheria procumbens* L.) collected in three different time periods (1^{st} – 15^{th} October 2006, 2^{nd}-30^{th} October 2006 and 3^{rd}-15^{th} March 2007). Samples of Eastern teaberry fruits weighing 5 g each were prepared and stored frozen until analysis.

After defrosting, the fruits were subjected to liquid-solid extraction, for which citric acid of pH 2 was used as a solvent. The extracts were prepurified by filtering through Whatman No 1 filter paper. The content of total phenols in raw extracts was determined according to FOLIN-CIOCALTEAU procedure, and expressed in terms of gallic acid (SINGLETON et al. 1999). The content of anthocyanins in the extracts (re-calculated as cyanidin 3-glucoside) was determined according to the methods designed by NIKETIĆ-ALEKSIĆ, HRAZDINA (1972) (method H) and WROLSTAD (1976) (method W). Antioxidative activity was established with an aid of the method suggested by YEN, HUNG (2000), which consisted of the determination of scavenging synthetic DPPH• (1,1-diphenyl-2-picrylhydrazyl) radicals in extracts from Eastern Teaberry fruits. The results were cited as inhibition percentages.

Extracts from Eastern teaberry fruits were mineralized in the so-called Teflon bomb using microwave energy. For this purpose, 1 cm^3 of each extract and 2 cm^3 of concentrated nitric acid (HNO_3) were used. After the mineralization, the extracts were transferred quantitatively to measuring flasks, which were filled to the full capacity with deionized water. The content of bioelements was determined using atomic absorption spectrometry (AAS) with a Unicam 939 device.

All the reagents for the above determinations were of analytical purity. The results of the chemical analyses, with three replications, underwent statistical processing for one-factor experiments, using Duncan's test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Our comparison of the content of total phenolic compounds in extracts from Eastern teaberry fruits harvested in three different times periods revealed statistically significant differences (Table 1). The content of total phe-

Table 1

Content of polyphenols (mg 100 g^{-1} fresh matter) and antioxidative activity (%) in *Gaultheria procumbens* extract fruits

Harvest date	Total phenols	Anthocyanins				Phenols other than anthocyanins				Antioxidative activity	
		method H		method W		method H		method W		inhibition	
	mean x	mean x	SD	mean x	SD	mean x	SD	mean x	SD	mean x	SD
I	360.9 ^{b}	18.25 ^{b}	1.00	10.63 ^{b}	0.79	342.69 ^{b}	72.09	350.31 ^{b}	71.69	87.55 ^{a}	2.01
II	495.2 ^{a}	21.47 ^{a}	0.76	13.11 ^{a}	1.35	473.73 ^{a}	45.97	482.09 ^{a}	45.08	89.28 ^{a}	0.40
III	180.0 ^{c}	17.36 ^{c}	0.20	7.83 ^{c}	0.88	162.64 ^{c}	8.92	172.17 ^{c}	6.38	85.88 ^{a}	1.36

SD – standard deviation;
Means followed by the same letters in columns did not differ significantly at $p < 0.05$ according to Duncan test.

nols in Eastern teaberry fruit extracts was the highest on the 2^{nd} harvest date and the lowest – on the 3^{rd} one. Similar dependence could be observed regarding the concentration of anthocyanins in Eastern teaberry fruit extracts. Irrespective of the determination method applied, the content of anthocyanins was the highest in extracts from Eastern teaberry fruits harvested on the 2^{nd} and the lowest – on the last, 3^{rd} harvest date. The content of phenols other that anthocyanins in the extracts was correlated with the total phenolic concentration and the content of anthocyanins. Likewise, it was the highest for extracts from fruits collected on the 2^{nd} harvest date and the lowest – for the 3^{rd} one.

Fruits of Eastern teaberry collected on the 2^{nd} harvest day had reached full maturity, which is why extracts obtained from these fruits contained the highest levels of total phenols and anthocyanins. Similar results were reached while examining fruits of black mulberry (Pliszka et al. 2007).

A close relationship has been found in the current study between the content of total phenols and anthocyanins in fruit extracts. Positive correlation was determined between the total phenolic content and anthocyanins in extracts from Eastern teaberry determined with the different methods. High correlation was obtained for the extracts obtained by method W ($R^2 = 0.871$), whereas the correlation for the extracts produced by method H was nearly two-fold smaller ($R^2 = 0.474$).

The content of polyphenolic compounds depends on many factors, such as: species of fruit, environmental conditions, agronomic treatments and fruit maturity (Asami et al. 2003, Kalt et al. 2001, Ważbińska et al. 2006). Collecting Eastern teaberries on different dates, the ripeness phase was taken into consideration. On the 2^{nd} harvest date the maturity of the fruits was optimum, therefore the extracts contained the highest levels of polyphenols (total polyphenols and anthocyanins).

Similar dependences were confirmed for black mulberries (*Morus nigra*) harvested in full maturity, in which the content of polyphenols was also the highest (PLISZKA et al. 2007). The concentration of polyphenols in fruits depends on a plant species. However, it needs to be mentioned that extracts from Eastern teaberries contain more total phenols and less anthocyanins than extracts from black mulberries, which have more anthocyanins than total phenols (PLISZKA et al. 2007).

Our analysis of polyphenolic compounds has demonstrated that extracts from Eastern teaberries were characterised by high antioxidative activity (from 85.58 to 89.28%), which did not depend on the fruit maturity (Table 1). Likewise, antioxidative properties of polyphenolic compounds in black mulberries were not dependent on a harvest date (PLISZKA et al. 2007). Polyphenolic compounds in extracts from black elberberries, tested in another study, possessed very high antioxidative properties (82 to 89%), in which they were similar to Eastern teaberries (PLISZKA et al. 2005).

Another question examined in the present study has been the relationship between the content of anthocyanins and total phenols versus their antioxidative activity in extracts from Eastern teaberries. Very strong correlation was found between the content of anthocyanins and their total antioxidative activity in extracts from Eastern teaberries obtained with H method ($R^2 = 0.761$). In contrast, the correlation between the total phenols and antioxidative activity was very low for extracts obtained from Eastern teaberry fruits with method W ($R^2 = 0.436$). KAUR, KAPOR (2002) as well as KÄHKÖNEN et al. (2001) have shown that the effect produced by polyphenolic compounds in fruit extracts on their antioxidative activity is varied (R^2 from 0.3 to 0.9). In turn, HASSIMOTO et al. (2005) claim that there is no correlation whatsoever between the content of total phenols or the content of vitamin C and antioxidative activity of extracts, thus suggesting that antioxidative properties are a product of a combination of various compounds which produce synergistic or antagonistic effects.

The antioxidative activity of polyphenols is shaped by several factors, including the species and composition of fruits, the chemical structure of phenolic compounds or the extraction method used (PLISZKA et al. 2003, 2005, RICE-EVANS et al. 1996, ZHENG, WANG 2003).

The extracts we obtained from the Eastern teaberry were subjected to chemical analyses to determine the content of some macroelements (Ca, Mg) and microelements (Zn, Fe), which are essential for human health (WIELEBA, PASTERNAK 2001).

The determinations of Mg, Fe and Zn did not reveal any statistically significant differences between their contents in the extracts of Eastern teaberry harvested on the consecutive dates (Table 2). As for calcium, significant differences were found between the levels of this macroelement in the extracts from Teaberries and the harvest date. The highest content of calcium was determined in the extracts from Teaberries collected on the 2nd

Table 2

Content of bioelements in extracts from Eastern teaberries (*Gaultheria procumbens*) (mg 100 g^{-1} fresh matter)

Harvest date	Ca		Mg		Fe		Zn	
	mean *x*	SD	mean *x*	SD	mean *x*	SD	mean *x*	SD
I	15.61 b	1.87	14.39 a	0.24	3.29 a	0.51	1.69 a	0.12
II	19.42 a	1.34	14.95 a	0.04	2.71 a	0.00	1.57 a	0.72
III	13.51 b	0.77	13.33 a	1.82	3.53 a	1.56	1.48 a	0.06

Explanations, see Table 1

harvest day. On the other two harvest dates, the concentrations of calcium were similar and lower than on the 2^{nd} date.

Irrespective of the harvest date, the teaberry extracts contained more calcium than magnesium and more iron than zinc.

CONCLUSIONS

1. The content of polyphenolic compounds (total phenols, anthocyanins) in extracts from the Eastern teaberry fruits depended on the fruit harvest date. The highest content of polyphenols occurred in extracts from teaberries harvested in the full maturity phase.

2. Irrespective of the harvest date, extracts produced from Eastern teaberry fruits were characterised by very high antioxidative activity.

3. Teaberry extracts contained more calcium than magnesium and more iron than zinc.

4. The content of such bioelements as Mg, Fe or Zn (unlike Ca) in teaberry extracts did not depend on the harvest date.

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INFLUENCE OF SOIL FERTILIZATION ON CONCENTRATION OF MICROELEMENTS IN SOIL SOLUTION OF SANDY SOIL

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Abstract

The study was carried out to asses the influence of soil mineral fertilization, manure application and soil liming on changes in the concentration of microelements (B, Cu, Fe, Mn and Zn) in soil solution. The tests were performed on samples of sandy soil collected from long-term fertilization experiments being conducted on lessive soil of the granulometric structure of clayish sand. Soil solution was prepared with the suction method. The concentration of microelements in soil solution was determined by means of the ICP method. The results showed an increase in Fe, Mn, Zn and Cu and a decrease in B and Mo in soil solution when soil was fertilized with nitrogen. The concentration of microelements in soil solution was not significantly influenced when soil was fertilized with phosphorous and potassium. Liming had a significant effect on a decrease in the concentration of Fe, Mn, Zn and Cu and an increase in B and Mo in soil solution. Soil application of manure significantly increased the concentration of microelements in soil solution.

Key words: microelements, soil solution, mineral fertilization, manure, liming.

WPŁYW NAWOŻENIA NA STĘŻENIE MIKROELEMENTÓW W ROZTWORZE GLEBOWYM GLEBY LEKKIEJ

Abstrakt

Celem pracy była ocena wpływu nawożenia mineralnego oraz stosowania obornika i wapnowania na zmiany stężenia mikroelementów (B, Cu, Fe, Mn i Zn) w roztworze glebowym gleby lekkiej. Próbki glebowe pobrano z trwałego doświadczenia nawozowego prowadzonego na glebie płowej o składzie granulometrycznym piasku gliniastego lekkiego. Roz-

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twór glebowy pozyskiwano metodą podciśnieniową. W roztworze glebowym mikroelementy oznaczono metodą ICP. Stwierdzono, że w warunkach nawożenia azotem obserwuje się zwiększenie stężenia żelaza, manganu, cynku i miedzi oraz zmniejszenie stężenia boru i molibdenu w roztworze glebowym. Nawożenie fosforem i potasem nie wpływa istotnie na stężenie mikroelementów w roztworze glebowym.

Wapnowanie przyczynia się do zmniejszenia stężenia żelaza, manganu, miedzi i cynku oraz zwiększenia stężenia boru i molibdenu w roztworze glebowym, a pod wpływem stosowania obornika obserwuje się istotne zwiększenie stężenia mikroelementów w roztworze glebowym.

Słowa kluczowe: roztwór glebowy, nawożenie mineralne, obornik, wapnowanie, mikroelementy.

INTRODUCTION

Soil solution is the environment where most important chemical reactions take place. Being a soil liquid phase, it controls transfer of dissolved components throughout the whole soil profile. Soil solution is involved in the transport of pollutants in soil and plays a significant role in the transport of elements in ecosystems. However, the most important function of soil solution is its involvement in plant feeding, as it constitutes the main source of water and food elements for plants (CAMPBELL et al. 1989, WOLT 1994, ŁABĘTOWICZ 1995, PORĘBSKA 2003). For many years now, the concentration of elements in soil solution has been treated as the indicator of soil fertility (HOAGLAND et al. 1920). Analyses of the chemical content of soil solution provide important data on the influence of agricultural practices, such as mineral fertilization (CURTIN, SMILLIE 1983, ŁABĘTOWICZ 1995, SIMARD et al. 1988, SMAL 1999) on the condition of soil environment.

The aim of this study has been to assess the influence of mineral fertilization, application of manure and soil liming on changes in the concentration of microelements (B, Cu, Fe, Mn and Zn) in soil solution of sandy soil.

MATERIAL AND METHODS

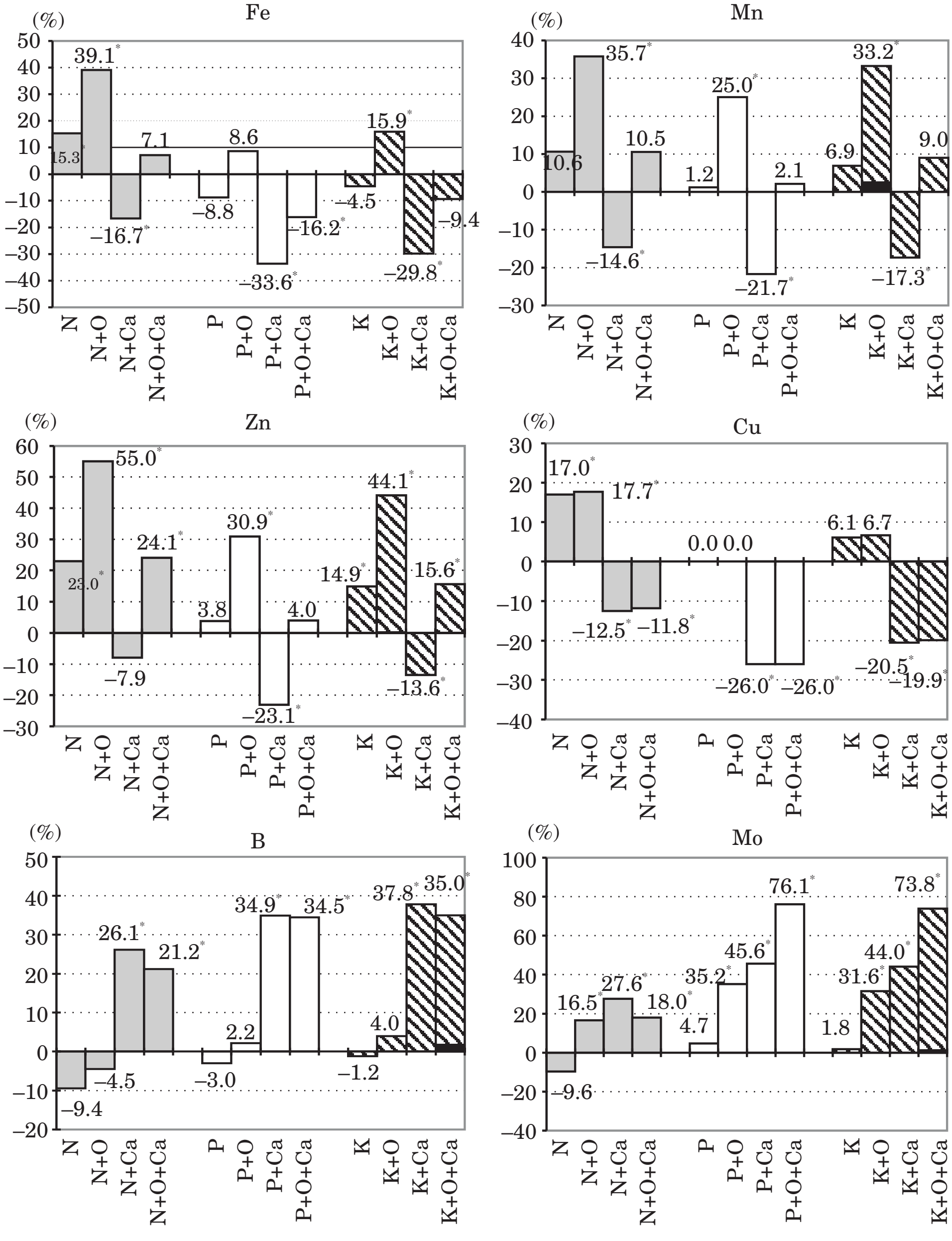
Assessments were carried out on soil samples collected from long-term fertilization experimental plots established in 1960 on lessive soil possessing granulometric features of clayish sand (classified by FAO as Albic Luvisols). The experiments encompass 16 objects being randomly fertilized by main components N, P, K, Ca with or without application of manure. Thus, the whole experiment included 32 fertilized objects with 4 replications. Potatoes, spring barley, rape (or mustard plant) and rye are cultivated in four-field crop rotation. Mineral fertilization was carried out at the following average rate (kg) per hectare: 140 N (ammonium nitrate), 50 P (triple superphosphate), 140 K (high percentage potassium salt). Liming (calcium carbonate) was applied every 4 years at a rate 1.14 Mg Ca per ha. Manure was applied at a rate 30 and 20 Mg per ha at each rotation of potatoes and rape (or mustard plant), respectively. Fertilization with microelements was not applied. Soil samples were collected from soil plough layer, then dried at 55°C. Soil solution was obtained with the suction method. The microelements: B, Cu, Fe, Mn, Mo and Zn were assessed with the ICP method. Evaluation of the influence of fertilization elements (N, P, K) applied when liming or manure were used was carried based on analyses of the main effects on the concentration of observed elements in soil solution as well as their interactions (expressed in %). The average value calculated from all combinations where a given fertilizer was not applied was treated as the zero level (0%).

RESULTS AND DISCUSSION

The results of the examination of the effects produced by the tested fertilizer compounds' on the concentration of microelements in soil solution showed a significant increase in the concentration of Fe, Mn, Cu and Zn in soil solution when soil was fertilized with nitrogen (Figure 1). The results on the effects of nitrogen fertilization on microelement uptake reported by other authors indicate that application of nitrogen fertilizers, and mainly ammonium nitrate, results in a decrease of soil reaction, which is associated with an increase of the concentration of microelements uptaken in the form of cations in soil solution (SANDERS 1982, 1983, CURTIN, SMILLIE 1983, SIMARD et al. 1988, SMAL 1999, ADAMS et al. 2001). An increase of solubility and mobility of many trace elements has been shown together with a decrease of pH by several authors (GĘBSKI 1998, GORLACH, GAMBUŚ 2000, BADORA 2002). MCBRIDE and BLASIAK (1979) showed that solubility of zinc decreased 30-fold per one unit of an increase of pH in a range of pH 5.0 – pH 7.0.

On the other hand, the concentration of boron and molybdenum in soil solution decreased with nitrogen fertilization (Figure 1). Under the conditions of lowered pH of sandy soil, the physicochemical adsorption of boron is reduced on particles of the solid soil layer, which enlarges losses of this element as a result of leaching down through the soil profile. As GOLDBERG and GLAUBIG (1986) reported, the maximum boron adsorption occurs at pH = 8.0-10.0, both on crystalline and amorphic oxides of iron and aluminum as well as on loamy minerals. The possibility of leaching of boron in the form of borane ions has not been called in question for a long time (ŚWIĘCICKI 1964).

Application of phosphorous fertilizers did not significantly influence the concentration of Fe, Mn, Zn and Cu in soil solution (Figure 1). The results



*N – nitrogen fertilization, P – phosphorus fertilization, K – potassium fertilization, Ca – liming, O – farmyard manure

Fig. 1. Effect of fertilization on changes of Fe, Mn, Zn, Cu, B and Mo concentration in soil solution. For the zero level (0%) an average was accepted from all fertilizing combinations where a given element was not applied

obtained by SIMARD et al. (1988) indicated the lack of association between the application of decreasing rates of phosphorous and concentrations of manganese and zinc in soil solution. However, LINDSAY and STEVENSON (1959a,b) showed that application of $Ca(H_2PO_4)_2$ onto acidic soils resulted in an increase of solubility of manganese, aluminum and iron when they are around superphosphate granules.

The effect of phosphorous fertilization on the concentration of molybdenum was not indicated in this study (Figure 1), however many authors have reported competition between molybdates and phosphates for location on soil particles in the solid phase (XIE, MCKENZIE 1991). This competition is caused by similar chemical properties of phosphorous and molybdenum in soil solution. Thus, there exists a theoretical possibility of the use of phosphorous fertilization in order to avoid molybdenum deficiency (SINGH, KUMAR 1979).

Soil liming significantly decreased the concentration of iron, manganese, zinc and copper as well as it caused an increase of boron and molybdenum in soil solution (Figure 1). The effect of liming on the concentration of microelements in soil solution was initially indicated by a change of soil reaction. Simard et al. (1988) showed that application of $CaCO_3$ resulted in a decrease of the concentration of manganese and zinc in soil solution. These authors suggest that the reason of such changes is binding of these elements by oxides and oxyhydroxides of aluminum and iron as well as sedimentation into less soluble forms. The results of SANDERS (1983) showed minor and irregular decreases in soil solution of the concentration of copper with an increase of pH due to the fact that copper almost always occurs in soil solution in a chelate form and a rate of formation of these complexes only to a small extent depends on soil solution. An increase of boron concentration in soil solution under soil liming could be caused by an increase of adsorption of this element on soil particles of the solid layer, which reaches the peak for boron at pH 8-10 (GOLDBERG, GLAUBIG 1985, 1986). Liming and a resulting increase of soil reaction causes enhancement of molybdenum mobility in soil (RILEY et al. 1987). At higher soil pH values there is a reduction of the amount of positive charges on soil colloids and an escalation of competition of molybdates and hydroxyl ions for adsorption areas on the particles of solid phase of soil. At the same time, the activity of iron and aluminum oxides able to adsorb molybdenum is decreased, which results in an increase of the concentration of this element in soil solution (REISENAUER et al. 1962, JARELL, DAWSON 1978). The decrease of the concentration of cations (iron, manganese, zinc and copper) observed in soil solution of the samples collected from the fertilization objects treated with lime was associated with an increase of the amount of free negative charges that were bound by cations from soil solution. On the other hand, an increase of the concentration of anions in soil solution that was observed as the effect of liming was associated with sedimentation of insoluble iron and aluminum molybdates as well as a decrease of the amount of positive charges on soil particles of the solid layer followed by an increased desorption of anions (boron, molybdenum, into soil solution (SIMARD et al. 1988).

Application of manure always resulted in an increase of the concentration of the investigated elements in soil solution (Figure 1). This was caused by introducing the elements with the mass of organic fertilizer followed by mineralization of the manure organic substance (CURTIN, SMILLIE 1983). An increase of the content of zinc, copper and iron in soil solution after manure application was also observed by DEL CASTILHO et al. (1993). These authors suggest that this phenomenon is due to chemical properties of soil (an increase of electric conductivity of the surface layer of soil, an increase of soil reaction, an increase of the content of easily soluble organic matter).

CONCLUSIONS

In the experiments discussed in this paper, mineral and natural fertilization was the factor that significantly determined the concentration of microelements in soil:

1. Among mineral fertilizers only nitrogen fertilization caused a decrease of soil pH and resulted in an increase of the concentration of iron, manganese, zinc and copper in soil solution while the concentration of boron and molybdenum was decreased. Fertilization with phosphorous and potassium did not significantly influence the concentration of microelements in soil solution.

2. Through neutralization of soil reaction, liming caused a decrease of the concentration of iron, manganese, zinc and copper in soil solution while the concentration of boron and molybdenum was increased.

3. A significant increase of the concentration of all investigated elements was observed in soil solution when manure was applied onto soil.

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CONCENTRATION OF MICRONUTRIENTS IN PEA AND LUPIN PLANTS DEPENDING ON THE SOIL TILLAGE SYSTEM

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Abstract

In a two-year field study, the changes in Cu, Zn, Mn and B concentrations in aerial parts of lupine and pea were observed under traditional and no-tillage system. In each year, plant material samples were collected four times at 10-day intervals, from 30 days after sowing to the flowering stage. In plants of one species but grown under different tillage systems, differences in the content of micronutrients occurred in the early growth season, and tended to disappear in the early inflorescence phase. In general, these concentrations were higher in plants under traditional tillage system. However, under drought conditions these concentrations were higher in plants under no-tillage system. Regardless of a tillage system, some changes in micronutrient concentration during the vegetation period were observed. Concentration of copper in aerial parts of plants evidently decreased as the plants grew older. Fluctuations in the levels of Zn, Mn, and B did not follow such clearly defined tendencies and were dependent on the plant's species and individual elements.

Keywords: no-tillage, pea, lupin, microelements in plants.

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ZAWARTOŚĆ MIKROELEMENTÓW W GROCHU I ŁUBINIE W ZALEŻNOŚCI OD SYSTEMU UPRAWY ROLI

Abstrakt

W 2-letnich badaniach polowych obserwowano zmiany koncentracji Cu, Mn, Zn i B w nadziemnej części łubinu i grochu uprawianych w tradycyjnym i zerowym systemie uprawy roli. W każdym roku pobierano próbki roślinne w 4 terminach, co 10 dni, w okresie od 30. dnia po siewie do fazy kwitnienia. W roślinach jednego gatunku, lecz rosnących w warunkach różnych systemów uprawy, wystąpiły różnice w poziomie zawartości mikroelementów w początkowym okresie wegetacji. Różnice te zanikały w fazie początku kwitnienia. Zawartość mikroelementów na ogół była wyższa w roślinach z uprawy tradycyjnej, natomiast w warunkach suszy – w roślinach z uprawy zerowej. W miarę upływu okresu wegetacji obserwowano pewne zmiany koncentracji mikroelemntów niezależnie od systemu uprawy roli. Zawartość Cu w częściach nadziemnych roślin wyraźnie malała w miarę zbliżania się do fazy kwitnienia. Zmiany zawartości Zn, Mn i B nie były tak jednoznacznie ukierunkowane i zależały od pierwiastka i gatunku rośliny.

Słowa kluczowe: uprawa zerowa, groch, łubin, zawartość mikroelementów w roślinach.

INTRODUCTION

Reduced soil tillage techniques have certain advantages over conventional tillage in that they prevent soil erosion, limit water loss and reduce crop cultivation costs. An extreme example of minimal tillage is zero-tillage, which involves direct sowing. In general, this technique generates lower crop yields than when soil is ploughed. This may be due to the altered physicochemical properties of unploughed soil, which in turn affect the uptake of nutrients by crops.

The aim of this study has been to compare the content of some micronutrients in vegetative organs of pea and lupine sown directly to unploughed soil and grown under conventional tillage as well as to trace modifications in the concentration of these elements during the crops' growth.

MATERIAL AND METHODS

The material for our study consisted of a collection of pea and lupine samples gathered in 2005-2006 from fields under no-till and conventional tillage systems. The NPK fertilization, adjusted to the requirements of both crops, was identical, regardless the tillage method applied. In each year, aerial parts of the plants were collected on four dates, at ten-day intervals, beginning on day 30 after the sowing and finishing during the early inflorescence stage. The plants were sown on 11th April 2005 and 24th April 2006. Six systematically distributed points (replications) were fixed on each 4 m^2

plot. On each of the sampling dates, whole aerial parts of plants were cut off from an area of 1 m^2. The samples were dry mineralized in a muffle furnace, diluted in hydrochloric acid and used for the following determinations: B content by the ICP method and Cu, Mn and Zn concentrations by the AAS method. For B and Mn determinations, certified material no NCSZC 76008 was used, whereas Cu and Zn determinations were referred to own material, i.e. plant samples IPE (Cu – IPE 06.2.4, Zn – IPE 06.2.4) from an interlaboratory research project in the Netherlands. The results are given as arithmetic means from 6 replications.

The soil samples, taken in early spring, underwent the following determinations: grain size distribution and concentration of available macro- and micronutrients extracted in 1 mol·dm^{-3} HCl. The properties of the soil arable horizon differred between the plots (Table 1). In general, the soil was sandy soils, containing little organic carbon, either acidic or very acidic in reaction.

Table 1

Characteristic of top layer of soil from tested fields

Field				C org. (g·kg^{-1})	pH 1 mol KCl·dcm^{-3}	Content (mg·kg^{-1})						
	1.0-0.1	0.1-0.02	<0.02			P	K	Mg	Cu	Mn	Zn	B
2005 Lupin	55	28	16	5.0	4.3	46	123	31	3.2	158	7.1	0.8
2005 Pea	57	26	17	6.5	4.4	49	159	33	3.3	186	7.2	0.9
2006 Lupin	36	40	24	6.0	5.0	58	152	43	3.0	166	8.1	0.6
2006 Pea	44	36	19	6.7	5.3	74	156	32	3.0	181	9.6	0.8

The weather during the period of plant sampling also varied from year to year. The humidity conditions over the given time period were characterized using Seljoninov's hydrothermal index (after STRNAD 1979), quoting its values in every ten-day period preceding each plant material collection (Figure 1). This index includes an average air temperature and total rainfall in a specific time period. When it falls below 1, it indicates drought (<0.3 – catastrophic drought), and when it goes above 2, it suggests excess water in soil. The supply of plants with water in 2006 was much worse than in 2005. In 2006, drought was observed in all the time intervals preceding the consecutive plant material samplings except the third date. The drought was particularly severe in the last ten days.

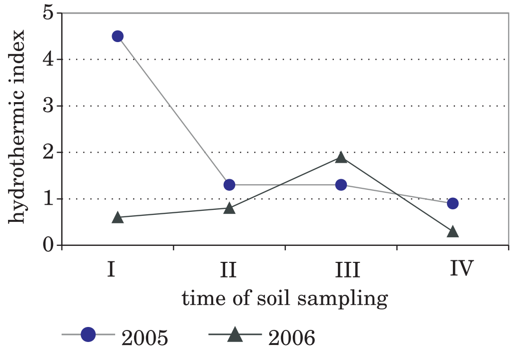
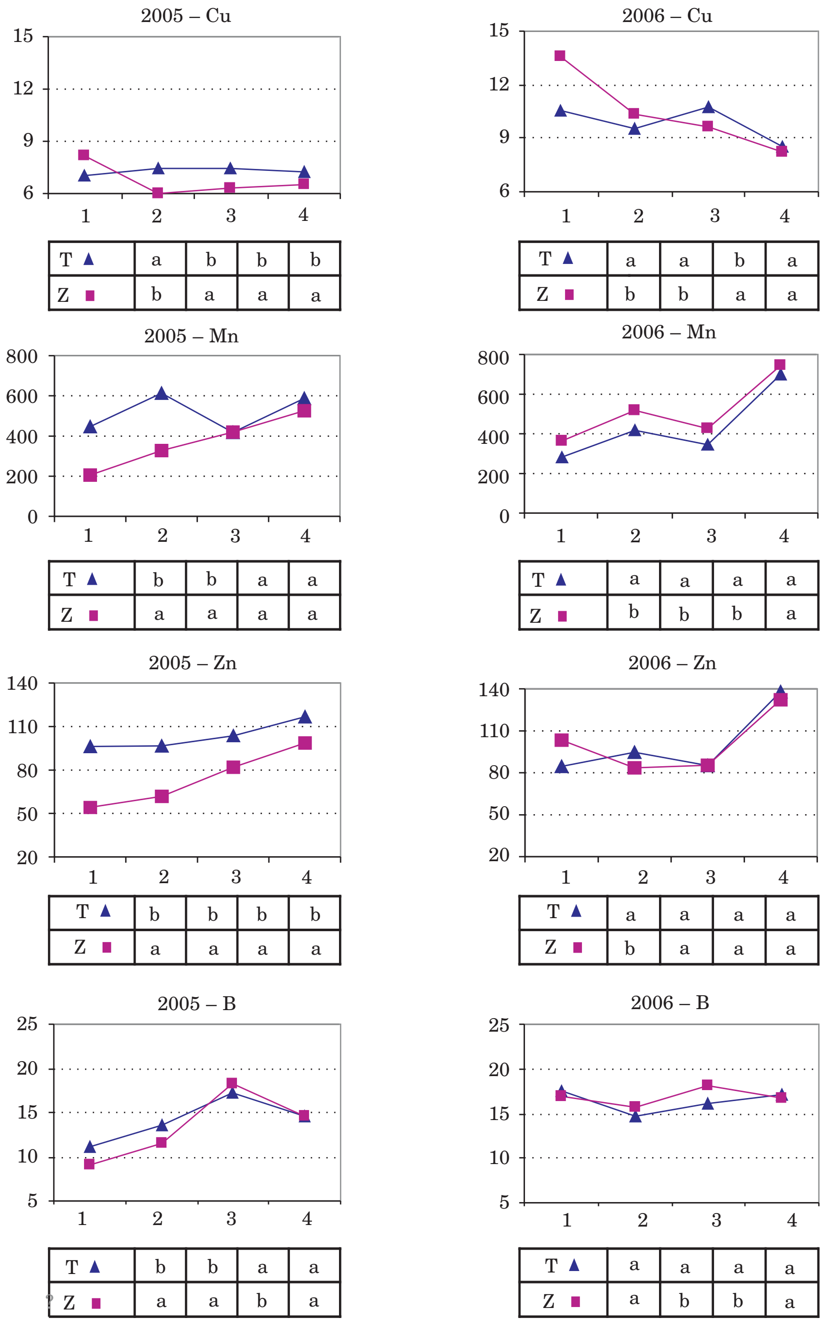


Fig. 1. Hydrothermics index values for 10-days periods

RESULTS AND DISCUSSION

The concentration of micronutrients in plants, during their vegetative growth to the inflorescence phase, was different between the two years of the study. The largest differences occurred in the amounts of copper. The content of Cu in lupine (Figure 2) and pea (Figure 3) was much higher in 2006 than in 2005, although the concentration of this element in soil was nearly identical in both years (Table 1). In 2005, the concentration of copper in plants ranged from 6.8 to 7.5 $mg \cdot kg^{-1}$ for lupine and from 6.4 to 8.0 $mg \cdot kg^{-1}$ for pea, whereas in the next year, 2006, it was 8.4-12.0 $mg \cdot kg^{-1}$ and 7.8-13.7 $mg \cdot kg^{-1}$, respectively. The content of Cu_{HCl} determined on a single occasion of soil sampling did not provide us with any information on the availability of this metal to crops over a longer period of time. It is most likely that the total pool of plant available forms of copper in 2006 was higher as the soil then contained more <0.1 mm fraction and organic carbon, which meant that its sorptive complex was greater than in 2005 (Table 1). The differences in the quantities of the other micronutrients between the years were smaller and less regular than those observed for copper. A higher level of Zn and Mn in pea was found in 2005, which may have been caused by a lower soil pH than in 2006; the content of B was higher in 2006.

Comparison of the concentrations of the micronutrients in both crops has demonstrated certain differences, too. They were the largest for manganese and smallest for boron. On the last sampling date, that is immediately before the inflorescence phase, the differences were much smaller or disappeared. The variation in the content of Mn was as high as 60% whereas that of boron ranged from less than 10 to 13%. Manganese is a particularly important microelement in nutrition of leguminous crops, especially pea,



2005 – Cu

T ▲	a	b	b	b
Z ■	b	a	a	a

2006 – Cu

T ▲	a	a	b	a
Z ■	b	b	a	a

2005 – Mn

T ▲	b	b	a	a
Z ■	a	a	a	a

2006 – Mn

T ▲	a	a	a	a
Z ■	b	b	b	a

2005 – Zn

T ▲	b	b	b	b
Z ■	a	a	a	a

2006 – Zn

T ▲	a	a	a	a
Z ■	b	a	a	a

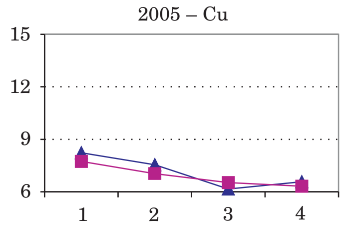
2005 – B

T ▲	b	b	a	a
Z ■	a	a	b	a

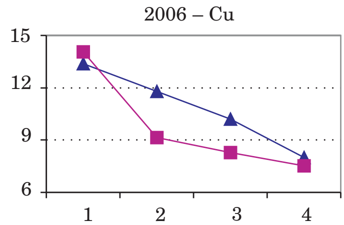
2006 – B

T ▲	a	a	a	a
Z ■	a	b	b	a

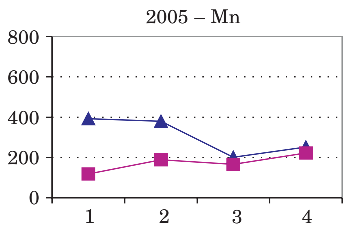
Fig. 2. Dynamics of the content of micronutrients (mg . kg^{-1}) in dry matter of aerial parts of lupine under conventional (T) and zero-tillage (Z) in ten-day intervals from day 30 after sowing to the onset of the inflorescence stage. There is no significant difference between the same letters in columns (method of tillage) according to Tukey's test (p<0.05)



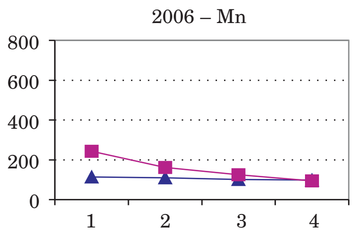
T ▲	a	a	a	a
Z ■	a	a	a	a



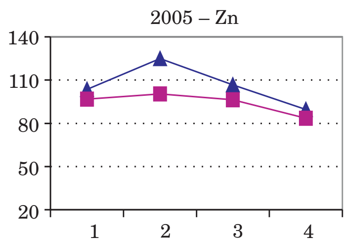
T ▲	a	b	b	a
Z ■	a	a	a	a



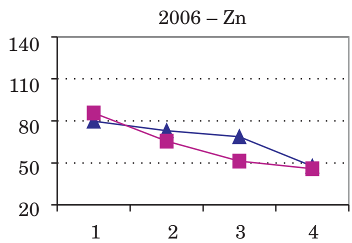
T ▲	b	b	a	a
Z ■	a	a	a	a



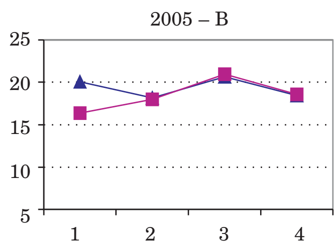
T ▲	a	a	a	a
Z ■	b	b	a	a



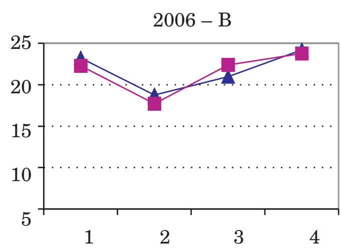
T ▲	a	b	a	a
Z ■	a	a	a	a



T ▲	a	a	b	a
Z ■	a	a	a	a



T ▲	b	a	a	a
Z ■	a	a	a	a



T ▲	a	a	a	a
Z ■	a	a	a	a

Fig. 3. Dynamics of the content of micronutrients (mg . kg^{-1}) in dry matter of aerial parts of pea under conventional (T) and zero-tillage (Z) in ten-day intervals from day 30 after sowing to the onset of the inflorescence stage. There is no significant difference between the same letters in columns (method of tillage) according to Tukey's test (p<0.05)

because these plants are highly intolerant to its deficit (KATYAL, RANDHAWA 1983). Consequently, the level of Mn can affect the volume of yields produced by legumes. In their study, YU, RENGEL (1999) found out that manganese deficit caused depressed green matter yield of lupine as early as in the initial growth phases of this crop.

KORZENIOWSKA and STANISŁAWSKA-GLUBIAK (2006) reported that different tillage methods caused differences in the nutrition of oats. Many authors report that the content of organic matter in soil as well as the concentration of N, P, K, Fe, Mn, Cu and Zn in the surface soil horizon are larger under no-till farming than when soil is ploughed (FRANZLUEBBERS, HOSS 1996, LAVADO et al. 1999, MARTIN-RUEDA et al. 2007), thus it can be expected that the concentration of nutrients in crops grown under reduced tillage should also be higher. Contrary to this, in our study sometimes the opposite was true. In 2005, lupine contained more micronutrients, especially Mn and Zn, when grown under conventional tillage than no-till systems. However, in the following year, lupine sown directly to unploughed soil contained more Cu, Mn and B. It seems that one possible reason is the differences in the humidity conditions observed between both years. ARSHAD et al. (1999) and PABIN et al. (2002) emphasize the positive influence that zero-tillage has on water accumulation in soil, especially in dry years. The drought observed during the first sampling dates in 2006 was less harsh on crops growing under minimal tillage than the ones sown to ploughed soil, which facilitated the uptake of micronutrients from unploughed soil. ZECH et al. (2000) demonstrated that the content of nutrients in tilled and non-tilled soil varied between the dry and wet seasons.

SANTIAGO et al. (2008) did not discover significant differences in the content of Fe, Cu and Zn in sorghum grown under traditional and zero-tillage systems, even though the soil content of available forms of nutrients was higher in non-tilled soil. However, unlike our tests, their study was carried out on alkaline soil, in which the availability of nutrients to plants is limited.

The course of the curves which illustrate the direction of the modifications in the concentrations of micronutrients in pea and lupine depended on a plant species, but was similar for both tillage methods. The vegetative parts of lupine were found out to contain decreasing levels of copper; in pea, both Cu and Zn tended to decline. In another study, performed under controlled conditions, PODLEŚNA and WOJCIESKA (1996) also observed declining levels of Cu and Zn in vegetative parts of pea. Changes in the concentration of other micronutrients were not so regular.

CONCLUSIONS

1. Changes in the concentration of micronutrients in vegetative parts of lupine and pea observed over the growth season were similar for both tillage methods. The concentration of Cu tended to decline, whereas the changes in the content of Mn, Zn and B were less regular and depended on the micronutrient and crop species.

2. The differences in the content of micronutrients in plant tissues, observed during this study, were the largest in the early vegetative growth and tended to disappear as the inflorescence stage was approaching.

3. Under drought conditions, levels of micronutrients were generally higher in crops grown under zero-tillage; when water supply was good, more micronutrients accumulated in crops cultivated traditionally, with soil ploughing.

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INFLUENCE OF MODIFIED TRANDERMAL HORMONE REPLACEMENT THERAPY, INCLUDING MAGNESIUM, ON BONE FORMATION MARKERS WITH OSTEOARTHROSIS OF SPINE IN WOMEN

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Abstract

In a randomized study 50 women, aged 51.7±2.8 years, suffering from primary osteoarthrosis (OA), were divided into two, equal groups (I, II). The women were employed in garment industry in contract work system. They were working in compulsory, mainly standing position. The women complained of backache of the lumbar region continuing for the minimum 5 years.

During the study, bone mineral density (BMD) of the lumbar spine was assessed twice with the densitometry DEXA method (Lunar Corporation equipment). Before treatment, structural changes in the lumbosacral spine were revealed using a CT Simens Sonata Plus 4. One energy technique (SEQCT) was applied. Concentrations of bone-forming markers in serum were measured three times: before treatment and 3 and 12 months afterwards. The concentration of acid phosphatase in serum was assessed by the enzymatic method according to Hitachi. The concentrations of osteocalcin and procolagen were radioimmunologically assessed by means of DRG Company – sets and concentration basal prolactin (PRL) before treatment radioimmunoassy kits produced by bioMerieux.

In the first stage of the treatment, the women in the first group received placebo for three months. Slow Mag B6 was administered for three months to the women in the second group.

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In the second stage of the treatment, the women in both groups received 21-day therapeutic cycles of modified transdermal hormonal replacement therapy. Additionally, bromocriptine (2.5 mg per day) and Slow Mag B6 (160 mg per day) were administered orally. The cycles repeated at a 7-day interval. During the interval, withdrawal bleeding occurred.

The results were statistically assessed by means of computerized programme package Statistica PL, version 5. It was stated that in 60% of women suffering from primary OA the basal concentration of prolactin in serum in was elevated above 25 ng/ml; in 25% women it was on the border level, and in 15% of the patients it was below the lower limit of the normal values. The combined treatment in women suffering from OA caused increase in bone-forming markers and decrease in pathological resorption processes of mineralization of the vertebral bodies. After 12 months of the therapy, resorption in the lumbar spine was diminished compared to the initial values, before the treatment. These changes were significant in L3/L4 vertebral bodies (p<0.05).

Key words: Osteoarthrosis. bone mineral density, bone-forming markers, hyperprolactinemia, modified hormonal replacement therapy.

WPŁYW ZMODYFIKOWANEJ PRZEZSKÓRNEJ HORMONOTERAPII ZASTĘPCZEJ I MAGNEZU NA STĘŻENIA MARKERÓW TWORZENIA KOŚCI U KOBIET ZE ZMIANAMI ZWYRODNIENIOWYMI KRĘGOSŁUPA

Abstrakt

Badaniem objęto 50 kobiet w wieku 51,7±2,8 lat z pierwotną osteoartrozą (OA), podzielonych na 2 grupy wg listy randomizowanej, zatrudnionych w przemyśle włókienniczym, w systemie pracy akordowo-potokowej, w pozycji wymuszonej, które od 5 lat uskarżały się na bóle w okolicy kręgosłupa lędźwiowego. Gęstość mineralną trzonów kręgów lędźwiowych oceniono 2-krotnie densytometrem, firmy Lunar Corporation, metodą DEXA, natomiast zmiany strukturalne kręgosłupa L1/S tomografem firmy CT Siemens Sonata Plus 4 techniką pojedynczej energii (SEQCT).

Stężenia markerów tworzenia kości określono 3-krotnie: przed leczeniem, po 3 i 12 miesiącach leczenia. Stężenie kostnej fosfatazy zasadowej oznaczono enzymatycznie, osteokalcyny i prokolagenu radioimmunologicznie zestawami firmy DRG. Podstawową prolaktynę (PRL) oznaczono przed leczeniem radioimmunologicznie zestawem bioMerieux. Kobiety z grupy I w pierwszym etapie leczenia przez 3 miesiące otrzymywały placebo, a w grupie II doustnie Slow Mag B6 w dawce 160 mg/24 h. Natomiast w drugim etapie kobiety z obu grup otrzymywały w 21-dniowym cyklu terapeutycznym zmodyfikowaną, przezskórną hormonoterapię zastępczą, bromokryptynę doustnie w dawce 2,5 mg/24 h i Slow Mag B6 w dawce 160 mg/24 h z przerwą 7-dniową w celu wystąpienia krwawienia. Analizy statystyczne z uzyskanych wyników przeprowadzono za pomocą pakietu Statistica PL, wersja 5. Stężenie prolaktyny podstawowej u 60% kobiet z OA wynosiło powyżej normy 25 ng/ml, u 25% kobiet górna granica normy, a u 15% kobiet poniżej dolnej granicy normy.

Zastosowanie skojarzonego leczenia u kobiet z OA wywarło pobudzający wpływ nie tylko na stężenia markerów tworzenia kości, ale również na wzrost aktywności osteoklastów pobudzających proces resorpcji nadmiernej mineralizacji trzonów kręgowych, która w 12-miesięcznej obserwacji ulegała zmniejszeniu w stosunku do wartości wyjściowych ze znamiennością w kręgu L3 i L4 (p<0,05).

Słowa kluczowe: osteonrtroza, gęstość mineralna, markery tworzenia kości, hiperprolaktynemia, zmodyfikowana hormonoterapia zastępcza.

INTRODUCTION

Osteoarthrosis (OA) is connected with diminished metabolism of bones, bone formation and resorption and increasing mineral density (BMD). Osteroarthrosis is a very frequent disease in human population, leading to disability (GOGGS et al. 2005) and associated with large costs of diagnosis and treatment (ANNUON et al. 200, SZCZEPAŃSKI 2000). Depending on its ethiopathogenesis, OA is divided into primary and secondary type. Primary OA is a genetic disease, which initially invades small joints of the upper limbs, and than major joints of the limbs. Secondary OA is a multifactorial disease, developing against the inflammatory (DELEO et al. 2001, Punki et al. 2005, YOSHIHARA et al. 2000, 2001) hormonal (STANOSZ et al. 2000) and traumatic (NIELSEN et al. 2008) background. Secondary OA is chronic and progressive. In the initial stages, arthritis and inflammatory reactions with inflammatory exudates are found. These early changes are dominated by inflammatory reactions connected with metaloproteinases (YOSHIIHARA et al. 2000) and cytokines (RAAP et al. 2000). Despite causing social concern, osteoarthrosis is far less known than other chronic diseases, such as hypertension arterials, diabetes mellitus, osteoporosis or neoplasms. Although there is a wealth of reports on the etiopathogenesis and diagnosis of OA, no unambiguous recommendations for its treatment have been established.

OBJECTIVE

The aim of the study has been to analyse the influence of transdemal modified hormonal replacement therapy (HRT) and the application of magnesium and dopaminergic agent (bromocriptine) on the concentrations of bone alkaline phosphatase, osteoclacine, procolagene in serum and on the bone mineral density (BMD) of the lumbar spine in women in the early perimenopausal phase.

MATERIAL AND METHODS

The study compromised 50 women with primary osteoarthrosis aged 51.7 ± 2.8 divided into two, equal, randomized groups (control I and experimental II groups). There were no differences between the groups in the age, body mass index, parity, place of living, habits and working conditions or the duration of the postmenopausal period. The main complaints were the lumbar backache and paresthesis. Bone mineral density (BMD L_2-L_4), determined at baseline (before treatment) and at 12 months, was assessed with a DEXA dual energy x-ray absorptiometry (Lunar Corporation DPX-IQ) scanner, which utilizes hydroxyapatite level in g/cm˛ as an expression of the degree of bone mineralization. The results of BMD L_2-L_4 were interpreted according to the WHO criteria. Before the treatment, structural changes in the lumbosacral spine were revealed using a CT Simens Sonate Plus 4. One energy technique (SEWCT) was applied. The concentrations of bone-forming markers: bone alkaline phosphatase, osteocalcine and procolagene in serum, were assessed. The concentration of bone alkaline phosphatase were assayed enzymatically (ROSAŁKI et al. 1984). The concentrations of osteocalcine and procolagene were assessed radioimmunologically by means of a DRC company sets. The concentrations of basal levels of prolactin (PRL I) were measured by means of bioMerioux commercial sets. Statistical calculations were performed using Statistica PL Package, version 5 (STANISZ 1988). The women of the control group (I), were receiving placebo for months. The women of the study group (II) were administered a commercial, pharmaceutical therapeutic agent, i.e. Slow Mag B_6, in the daily dose 160 mg/24 h for 3 months. Afterwards, both groups underwent modified transdermal hormonal replacement therapy (HRT): micronized 17â-estradiol (molar mass 272,39 g/mol) in the form of patches (Systen, Janssen-Cilag, Switzerland) with subsequently increasing and decreasing doses (25, 50, 75 and 75 ug/dose), imitating the physiological serum concentrations of estrogens throughout the therapeutic cycle (Stanosz et al. 2007), with concomitant oral intermittent progesterone administration (molar mass 314.47 g/mol); (Lutein Firm Adamed, Poland) in the second phase of the therapeutic cycle in doses of 50 mg daily for six days and subsequently 100 mg daily for the next six days during 12 months. Moreover, independently of the stage of the therapeutic cycle, both groups received a commercial, pharmaceutical agent containing magnesium and vitamin B_6, called Slow Mag B_6, which was administered orally in the dose of 160 mg/24h, and a dopaminegic agent, bromocriptine (manufacturered by Polfa), in the dose 2.5 mg/24 h.

RESULTS

The results of the study are compiled in Tables 1, 2 and Figure 1. Table 1 presents concentrations of bone alkaline phosphate, osteocalcine, procolagene in serum before and after 12 months of the combined treatment in both groups (I, II). There were no differences in the concentrations of bone-forming markers between the groups before the treatment. In study group (group II), receiving magnesium, a statistically significant increase in bone alkaline phosphatase ($p < 0.05$) and procolagene ($p < 0.05$) occurred compared to women receiving placebo (group I). After 12 months of the combined treatment, in both groups (I, II) the concentrations of bone-forming markers in

Table 1

Concentrations of bone-forming markers before and after combined treatment

Groups	n	Before study			After 3 months of treatment			After 12 months of treatment		
		bone alkaline phosphatase (U L^{-1})	osteocalcine (mg ul^{-1})	procolagene (mg ml^{-1})	bone alkaline phosphatase (U L^{-1})	osteocalcine (mg ul^{-1})	procolagene (mg ml^{-1})	bone alkaline phosphatase (U L^{-1})	osteocalcine (mg ul^{-1})	procolagene (mg ml^{-1})
I	25	18.4 ±4.1	11.4 ±3.1	115.4 ±12.2	19.7 ±3.1	16.7 ±8.4	125.4 ±9.1	21.4 ±5.7	21.7** ±15.1	121.7* ±15.1
II	25	20.3 ±3.2	13.2 ±4.3	109.6 ±15.3	30.7* ±6.1	20.3 ±9.7	132.9* ±8.1	25.2 ±9.7	25.1** ±17.3	125.1* ±17.3

*$p<0.05$
**$p<0.01$

Table 2

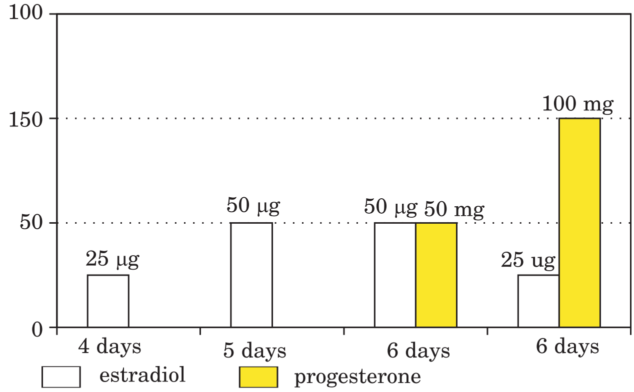
Bone mineral density of the lumbar spine L1-L4 (mg hydroxyapatite cm^{-2})

Groups	n	PRL (ng l^{-1})	Primary exam				Exams after 12 months of threat				Reference values
			L_1	L_2	L_3	L_4	L_1	L_2	L_3	L_4	
I	25	25.6 ±7.6	131 ±21.2	135 ±19.7	136 ±20.7	140 ±25.1	124 ±19.0	130 ±16.7	128* ±22.4	131.2* ±26.1	125 ±8.7
II	25	24.9 ±6.9	129.2 ±19.8	128.7 ±16.4	130.5 ±17.2	135.4 ±19.2	123.4 ±17.1	121.7 ±16.9	122.1* ±26.9	127.4* ±19.4	120.3 ±7.1

*$p<0.05$

serum were statistically higher in comparison with the initial values (Table 1). Table 2 contains results of the bone mineral density (BMD) of the lumbar spine L_1-L_4. There were no differences in the degree of mineralization of the lumbar spine between groups. However, after 12 months of the combined treatment, the degree of mineralization density decreased statistically significantly in vertebral bodies L_3 and L_4 ($p < 0.05$). Tomographic images of the lumbar sacral spine revealed in 15 women (30%) with OA the narrowing of intervertebral discs in L_1/ L_2 and L_2/L_3 with osteophytoses.

In 25 women (50%), herniae of annulus fibrosus of intervertebral discs without radiculalgia were found in the intervetebral spaces L_4/L_5 and L_5/S_1. In 10 women (20%), herniae of annulus fibrosus of intervertebral discs with compression of nerve roots causing radiculopathy were revealed in the intervetebral space L_5/S_1. Three months of Slow Mag B_6 administration in women of the study group caused significant diminishing of radiculopathy.



Pig. 1 The influence of modified transdermal hormone replacement in therapeutic cycle. Use of fluctuating doses hormones leads to obtaining physiological concentrations of estrogens and progesterone in serum

DISCUSSION

Magnesium is an intracellular bioelement essential for health. This macroelement is of great importance in physiological processes (ANNOUN et al. 2007, SZCZĘSNY 2002). Magnesium is an activator of many enzymes participating in energy processes, influencing concentration of other electrolytes by enhancing their capacity for assimilation (HUNTER et al. 2003). Children, elderly people, patients with hypertonia arterials, circulation insufficiency and the digestive system diseases are at risk of magnesium deficiency (ANNOUN et al. 2007). Administration of magnesium, in the present study, caused the activation of bone alkaline phosphatase (p 3< 0.05) in serum. In patients with OA, after this therapy, other metabolic processes occurring in bones were activated (IMADA et al. 2003, JIANG et al. 2008, TANIMOTO et al. 2004). The decreased metabolism in bones of patients with OA is caused not only by advanced age (SZCZEPAŃSKI 2000, SZCZĘSNY 2002), but also by enzymatic (YOSHIHARA et al. 2000), inflammatory (DELEO et al. 2001, PUNZI et al. 200) cytokines (RAAP et al. 2000, YOSHIHARA et al. 2004) and traumatic (NIELSEN et al. 2008) agents. Among hormonal factors involved in the development of OA, hyperprolactinemia, which is found in 86% of the studied women, seems very important (STANOSZ et al. 2000). Prolactin directly exerts inhibits osteoblasts and osteoclastic activity. Indirect influence of prolactin on bone metabolism, due to disturbances in the conversion of androgens into estrogens, has been demonstrated. The combined treatment discussed in this paper (magnesium plus dopaminegric agent) stimulated bone metabolism in women with OA, which was revealed as a significant increase in the concentration of bone alkaline phosphatase ($p < 0.05$) and osteocalcine ($p < 0.05$) in serum. Moreover, after 12 months of the combined treatment the concentrations of bone-forming markers were significantly higher than before treatment. In women with spondyloarthrosis of the spine, an increase in BMD L_1-L_5 in comparison with reference values of the densitometer was found. It was caused not only by more intense mineralization of the vertebral bodies and ligaments (STANOSZ et al. 2000), but also by the mineralization of intervertebral discs (GARNELO et al. 2005, TANIMOTO et al. 2004, WANG et al. 2005) and the spinous processes (GOLDBERG 2000). Application of the combined treatment in women with OA demonstrated a favourable effect on the resorption process of vertebral bodies after 12 months of the treatment, accompanied by decreasing BMD of vertebral bodies compared to the initial values but, but significant results occurred only in vertebral bodies L_3 and L_4 ($P < 0.05$).

CONCLUSIONS

1. The stimulatory effect of the pharmacological therapeutic agent Slow Mag B_6 on bone formation processes occurs through significant increase of bone alkaline phosphatase and procolagene.

2. Frequent occurrence of hyperprolactonemia in women with osteoarthrosis suggests need to add a dopaminergic agent (bromocriptine) to the combined treatment of this chronic disease.

3. Administration of modified transdermal hormonal replacement therapy influences bone forming as well as bone resorption processes.

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CONCENTRATIONS OF SOME MACROELEMENTS IN POTATO TUBERS STORED AT 4°C AND 8°C

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Abstract

The objective of the investigations was to evaluate changes in concentrations of total protein (nitrogen x 6.25), phosphorus and potassium in tubers of two potato cultivars: Rywal and Saturna, as dependent on the application of herbicides (Afalon 50 WP, Azogard 50 WP, Sencor 70 WG, and Apyros 75 WG), after harvest and after 3 and 6 months of storage. Concentrations of total protein, phosphorus and potassium in potato tubers were strongly conditioned by genetic features of the cultivars. The tubers collected from the objects sprayed with the herbicides over the vegetation period contained by 3.7%, 8.1%, and by 3.5% more protein, phosphorus and potassium, respectively, than those nursed exclusively mechanically. The corresponding values in the latter case reached respectively (means for the objects with the use of herbicides) 24.4 g kg^{-1}, 660.5 mg kg^{-1} and 5351.3 mg kg^{-1}. After 3 and 6 months of storage at 8°C, the content of nitrogen was significantly lower. Similarly, tubers stored at 4°C contained significantly less nitrogen, but not earlier than after 6 months. In contrast, concentrations of phosphorus and potassium did not change significantly over the storage of tubers in chambers with the lower temperature (4°C).

Key words: potato, protein, phosphorus, potassium, herbicides, storage.

ZAWARTOŚĆ WYBRANYCH MAKROELEMENTÓW W BULWACH ZIEMNIAKA PRZECHOWYWANYCH W TEMPERATURZE 4 I 8°C

Abstrakt

Celem badań było określenie zmian zawartości białka ogólnego (azot x 6,25), fosforu i potasu w bulwach ziemniaka odmian Rywal i Saturna w zależności od zastosowanych herbicydów (Afalon 50 WP, Azogard 50 WP, Sencor 70 WG, Apyros 75 WG), po zbiorze i po

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3 i 6 miesiącach przechowywania. Zawartość białka ogólnego, fosforu i potasu w bulwach ziemniaka różnicowały istotnie uwarunkowania genetyczne odmian. Bulwy zebrane z obiektów opryskiwanych herbicydami w okresie wegetacji roślin zawierały o 3,7% więcej białka, o 8,1% fosforu, o 3,5% potasu niż bulwy roślin pielęgnowanych wyłącznie mechanicznie, dla których wartości te wynosiły odpowiednio (średnio dla obiektów opryskiwanych herbicydami): 24,4 g kg^{-1}, 660,5 mg kg^{-1} i 5351,3 mg kg^{-1}. Po 3 i po 6 miesiącach przechowywania w temp. 8°C zawartość azotu istotnie się zmniejszała. Również w bulwach przechowywanych w temp. 4°C poziom azotu istotnie się zmniejszył, lecz dopiero po 6 miesiącach. Natomiast zawartość fosforu i potasu nie zmieniała się istotnie podczas przechowywania w bulwach składowanych w komorach o niższej temperaturze (4°C).

Słowa kluczowe: ziemniak, białko, fosfor, potas, herbicydy, przechowywanie.

INTRODUCTION

Despite a wide selection of other raw products and foodstuffs on the market, potato tubers remain one of the staple elements of the Polish diet. In Poland, the intake of potatoes reaches about 120 kg per capita annually. Continues to be the source of many valuable components, such as highly nutritional protein, rich in exogenic amino acids (Mazurczyk 2005), and mineral compounds, including phosphorus and potassium (Leszczyński 2000, Kolasa 1993, Niederhauser 1993). The quality of raw potato is affected by several factors, such as agronomic practice, including weeding (Kraska 2002). Weeding is so important because competition with weeds for light and nutrients may cause lower concentration of some components in the tubers. In contrast, successful eradication of weeds promotes the yield potential of the cultivar and preserves its quality parameters (Głuska 2000). During the storage of tubers, especially at temperatures below 8°C, the content of protein and mineral compounds should not change considerably. However, in her investigations Rogozińska (1989) found losses of total protein after storage, while Pobereżny (2005) observed some changes in phosphorus and potassium concentrations during a 6-month storage of tubers.

The objective of the investigations was to evaluate changes in protein, phosphorus and potassium concentrations in potato tubers of cv. Rywal and Saturna after harvest, and 3 and 6 months of storage as affected by herbicides used for weed control.

MATERIAL AND METHODS

Tubers of two potato cultivars from field experiments carried out in 2002-2004 at Mochełek Experimental Station of the Bydgoszcz University of Technology and Life Science, Poland, were used for the investigations. The field experiments were designed as randomized sub-blocks, where the first experimental factor was the nursing treatments (plots without herbicides, treated with Afalon 50 WP, Azogard 50 WP, Sencor 70 WG, and Apyros 75 WG), while the second factor was the cultivars: the medium late potato cultivars Rywal and Saturna. Farmyard manure was used in autumn in the dose 25 t ha^{-1}, while mineral fertilizers were applied in spring before the potato planting in the amounts calculated according to the needs of the plant and the soil nutrient resources: nitrogen – 120 kg $N \cdot ha^{-1}$, phosphorus – 110 kg $P_2O_5 \cdot ha^{-1}$, and potassium – 120 kg $K_2O \cdot ha^{-1}$. The samples were taken after the harvest and than placed in storage chambers at 4°C and 8°C and relative air humidity of 95%. After the harvest and the two storage periods the tubers were analyzed for nitrogen (calculated for protein concentration Nx6,25), phosphorus and potassium. The results were evaluated statistically using the variance analysis according to the design of the experiment. The smallest significant difference was calculated with the use of Tukey's test.

RESULTS AND DISCUSSION

Owing to their nutritional value, potato tubers have an important role in human nutrition, for example they are a good supply of protein. Protein from potato tubers is very useful in anabolic processes, which means that their biological value is very high compared with soybean protein, and only slightly lower than the nutritional standard accepted for chicken egg protein (Mazurczyk 2005). The herbicides used in the experiment significantly increased the total crude protein content by 3.7% in tubers of both cultivars (mean 24 4 g kg fresh weight) in relation to tubers from the control plots (Figure 1). Similar results were reported by Kłosińska-Rycerska et al. (1979, 1975), Kołpak et al. (1987), Ceglarek et al. (1990), Banaszkiewicz (1993), and Zarzecka et al. (2000). The accumulation of proteins is also conditioned by genetic factors (Woda- Leśniewska 1993). Out of the two examined cultivars, cv. Saturna, had significantly more protein than the other one, cv. Rywal (Figure 1).

The results have shown that the content of the macroelements was modified by the experimental factors. The tubers from plants sprayed with the herbicides contained significantly more phosphorus (by 8.1%) than those harvested from the plots treated mechanically (mean for the objects with herbicides was 660.5 $mg \cdot kg^{-1}$). A similar tendency was observed as an increased concentration of potassium in the tubers from plants weeded both mechanically and chemically versus plants treated only mechanically (mean concentration of potassium in tubers from the objects with herbicides was 5351.3 $mg \cdot kg^{-1}$) – Figure 2. Higher content of macroelements in tubers can be explained by the fact that the plants free from weeds had better access to light, water and nutrients. Ceglarek, Księżak (1992) did not find any sig-

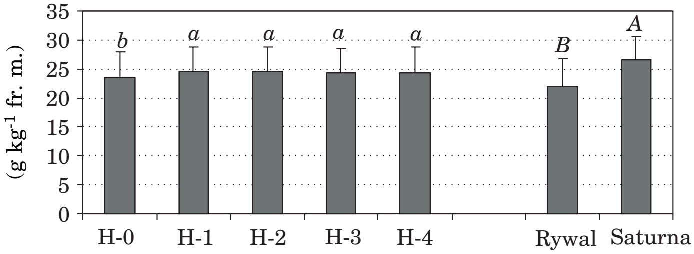
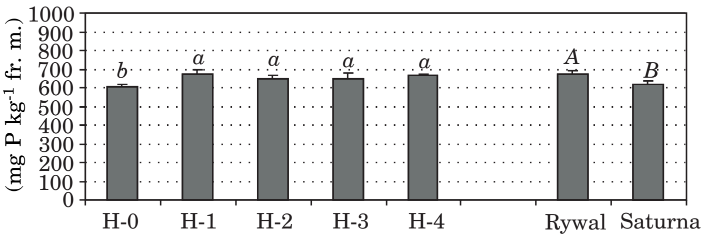
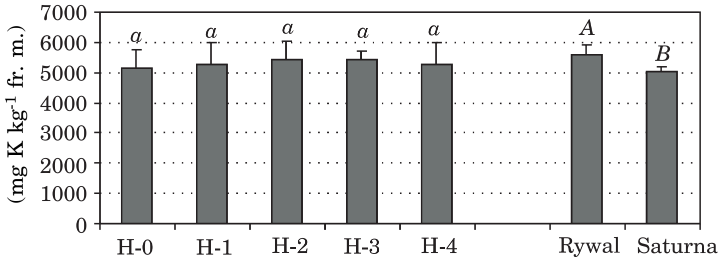


Fig. 1. Content of protein in the fresh weight of tubers (g kg^{-1}) of the potato cultivars depending on the herbicides used:
A, B, a, b – homogenous groups (no significant difference between the same groups)
H-0 – plots without herbicide, H-1 – herbicide Afalon 50 WP, H-2 – herbicide Sencor 70 WG, H-3 – herbicide Apyros 75 WG, H-4 – herbicide Azogard 50 WP



Explanation: H-0, H-1, H-2, H-3, H-4, A, B, a, b – see Fig. 1
Fig. 2. Content of phosphorus in the fresh weight of tubers (g kg^{-1}) of the potato cultivars depending on the herbicides used

nificant changes in concentrations of phosphorus and potassium in tubers harvested from objects nursed by the mechanical and chemical method. Kołpak et al. (1987) noted a small tendency of increasing concentrations of phosphorus and potassium in tubers under such conditions, while Zarzecka (1997) reported contrary results. Moreover, concentrations of phosphorus and potassium were significantly differentiated by genetic features of the cultivars (Figure 1), an observation that has also been demonstrated by Mikos-bielak, Sawicka (1999), Kolbe (1997), Wojnowska et al. (2000). Significant differences among cultivars in concentrations of potassium were also reported by Pobereżny (2005).



Explanations: H-0, H-1, H-2, H-3, H-4, see Fig. 1

Fig. 3. Content of potassium in the fresh weight of tubers (mg kg^{-1}) of the potato cultivars depending on the herbicides used

The content of the macroelements in tubers stored at 4°C and 8°C was significantly different for both cultivars (Table 1, 2, and 3). The cultivar Saturna accumulated more protein and less phosphorus and potassium than cv. Rywal. Similarly to the findings of ROGOZIŃSKA (1989), after 3 and 6 months in storage chambers at 8°C, tubers of both cultivars contained on average less protein. However, storage in chambers at 4°C significantly decreased the protein content after 6 months. Concentrations of phosphorus and potassium increased significantly during storage at the higher temperature. A small increase of concentrations of both elements after storage was observed also by POBEREŻNY (2005). It can be explained as a higher concentration of dry matter caused by decreasing amount of water used up by more intense life processes (transpiration, respiration) occurring during storage, especially at a higher temperature SOWA-NIEDZIAŁKOWSKA (1999, 2000). In our study, concentration of phosphorus in potato tubers did not change during storage at 4°C, although some changes were observed after 6 months. The results showed significant correlation between the period of storage and a specific herbicide. The tubers collected from the plots treated with Azogard 50 WP after 3 month of storage contained less phosphorus than after the harvest, afterwards the concentration of this macroelement increased but was never higher than the starting value. A possible explanation can be an individual reaction of a cultivar to the active component of the herbicide (prometrin), which can be an activator of biochemical changes occurring during storage.

According to the Institute of Foodstuffs and Nourishment in Warsaw (ZIEMLANSKI et al. 1995, ZIEMLANSKI (Ed.) 2001, GERTIG, PRZYSŁAWSKI 2006) the Recommended Daily Allowance for an adult human of about 70 kg equals 56 g of protein, 800 mg of phosphorus, and 3500 mg of potassium. Assuming that the losses of those elements during cooking of potato are negligible, consumption of about 300g of potatoes should cover 48.6% of the daily needs of adults for potassium, 25.1% for phosphorus 12.7% for protein (Nx6.25) (Table 4).

Table 1

Content of protein in fresh weight of tubers (g kg^{-1}]) of the potato cultivars depending on the herbicides used and the storage time at the temperature of 8°C and 4°C

Cultivars	Herbi-cides	After harvest	Time of storage					
			at the temperature of 8°C			at the temperature of 4°C		
			3 months	6 months	mean (3,4,5)	3 months	6 months	mean (3,7,8)
1	2	3	4	5	6	7	8	9
Rywal	H-0	21.1	20.5	20.0	20.5	20.7	20.5	20.8
	H-1	22.1	21.7	21.0	21.6	21.9	21.4	21.8
	H-2	22.2	21.7	21.0	21.6	21.9	21.4	21.8
	H-3	22.0	21.7	21.0	21.6	21.9	21.4	21.8
	H-4	22.0	21.4	21.0	21.5	21.7	21.4	21.7
Mean		21.9	21.4	20.7	21.3	21.7	21.2	21.6
Saturna	H-0	25.9	25.0	24.3	25.1	25.5	25.0	25.5
	H-1	26.8	26.0	25.5	26.1	26.4	26.2	26.5
	H-2	26.8	26.2	26.0	26.3	26.4	26.2	26.5
	H-3	26.5	26.0	25.7	26.1	26.2	26.0	26.2
	H-4	26.8	26.2	25.7	26.2	26.7	26.2	26.6
Mean		24.2	26.0	25.5	25.2	26.2	26.0	25.5
Mean for cultivars	H-0	23.5	22.9	22.1	22.8	23.1	22.6	23.1
	H-1	24.5	23.8	23.3	23.9	24.3	23.8	24.2
	H-2	24.5	23.8	23.6	24.0	24.3	23.8	24.2
	H-3	24.3	23.8	23.3	23.8	24.0	23.6	24.0
	H-4	24.4	23.8	23.3	23.8	24.3	23.8	24.2
Mean		24.2	23.6	23.1	23.6	24.0	23.6	23.9

$LSD_{=0.05}$ for the storage temperature:	8°C	4°C
herbicides used	n.s.	n.s.
cultivars	0.2	0.2
storage time	0.2	0.5
herbicides × cv	n.s.	n.s.
cv × herbicides	n.s.	n.s.
storage time × cv	n.s.	n.s.
cv × storage time	n.s.	n.s.
storage time × herbicides	n.s.	n.s.
herbicides used × storage time	n.s.	n.s.
cultivars × herbicides used × storage time	n.s.	n.s.

Explanations: H-0, H-1, H-2, H-3, H-4 – see Fig. 1, n.s. – non-significant difference

Table 2

Content of phosphorus in the fresh weight of tubers (mg kg^{-1}) of the potato cultivars depending on the herbicides used and the storage time at the temperature of 8ºC and 4ºC

Cultivars	Herbi-cides	After harvest	Time of storage					
			at the temperature of 8ºC			at the temperature of 4ºC		
			3 months	6 months	mean (3,4,5)	3 months	6 months	mean (3,7,8)
1	2	3	4	5	6	7	8	9
Rywal	H-0	631	738	749	706	721	718	690
	H-1	752	750	755	752	725	737	738
	H-2	683	686	687	685	697	711	697
	H-3	640	642	699	660	674	682	665
	H-4	707	676	693	692	670	676	684
Mean		683	698	717	699	697	705	695
Saturna	H-0	583	636	645	621	671	721	658
	H-1	594	631	648	624	625	636	618
	H-2	619	633	637	630	606	602	609
	H-3	660	668	695	674	631	675	655
	H-4	629	602	607	613	612	649	630
Mean		617	634	646	632	629	657	634
Mean for cultivars	H-0	607	687	697	664	696	720	674
	H-1	673	691	702	688	675	687	678
	H-2	651	660	662	658	652	657	653
	H-3	650	655	697	667	653	679	660
	H-4	668	639	650	652	641	663	657
Mean		650	666	682	666	663	681	665

LSD$_{=0.05}$ for the storage temperature:	8ºC	4ºC
herbicides used	n.s.	n.s.
cultivars	60	37
storage time	26	n.s.
herbicides × cv	n.s.	n.s.
cv × herbicides	n.s.	n.s.
storage time × cv	n.s.	n.s.
cv × storage time	n.s.	n.s.
storage time × herbicides	11	27
herbicides used × storage time	12	9
cultivars × herbicides used × storage time	n.s.	n.s.

Explanations: H-0, H-1, H-2, H-3, H-4 – see Fig. 1, n.s. – non-significant difference

Table 3

Content of potassium in the fresh weight of tubers (mg kg^{-1}) of the potato cultivars depending on the herbicides used and the storage time at the temperature of 8°C and 4°C

Cultivars	Herbi-cides	After harvest	Time of storage					
			at the temperature of 8°C			at the temperature of 4°C		
			3 months	6 months	mean (3,4,5)	3 months	6 months	mean (3,7,8)
1	22	3	4	5	6	7	8	9
Rywal	H-0	5510	5690	5870	5690	5600	5640	5583
	H-1	5640	5870	6200	5903	5650	5810	5700
	H-2	5750	6260	6420	6143	5690	5990	5810
	H-3	5600	5690	6100	5797	5830	6010	5813
	H-4	5520	6020	6190	5910	6560	6580	6220
Mean		5604	5906	6156	5889	5866	6006	5825
Saturna	H-0	4820	5100	5500	5140	5440	6070	5443
	H-1	4930	5040	5740	5237	5550	5830	5437
	H-2	5090	5400	5550	5347	5180	5520	5263
	H-3	5230	5350	5610	5397	6050	5870	5717
	H-4	5050	5330	5540	5307	5310	5580	5313
Mean		5024	5244	5588	5285	5506	5774	5435
Mean for cultivars	H-0	5165	5395	5685	5415	5520	5855	5513
	H-1	5285	5455	5970	5570	5600	5820	5568
	H-2	5420	5830	5985	5745	5435	5755	5537
	H-3	5415	5520	5855	5597	5940	5940	5765
	H-4	5285	5675	5865	5608	5935	6080	5767
Mean		5314	5575	5872	5587	5686	5890	5630

$LSD_{=0.05}$ for the storage temperature:	8°C	4°C
herbicides used	n.s.	n.s.
cultivars	450	390
storage time	250	n.s.
herbicides × cv	n.s.	n.s.
cv × herbicides	n.s.	n.s.
storage time × cv	n.s.	n.s.
cv × storage time	n.s.	n.s.
storage time × herbicides	n.s.	n.s.
herbicides used × storage time	n.s.	n.s.
cultivars × herbicides used × storage time	n.s.	n.s.

Explanations: H-0, H-1, H-2, H-3, H-4 – see Fig. 1, n.s. – non-significant difference

Table 4

Daily covering of the demand of adult man (weighing 70 kg) for proteins, phosphorus, potassium, assuming consumption of 300 g potatoes and negligible losses during culinary processing

Date of analysis	Daily intake			% covering day demand		
	protein (g)	phosphorus (mg)	potassium (mg)	protein	phosphorus	potassium
After 3 months storage at 8°C	7.08	200	1673	12.6	25.0	47.8
After 3 months storage at 4°C	7.20	199	1706	12.9	24.9	48.7
After 6 months storage at 8°C	6.93	205	1762	12.4	25.6	50.3
After 6 months storage at 4°C	7.08	204	1767	12.6	25.5	50.5

CONCLUSIONS

1. Herbicides applied during cultivation of potato significantly increased concentrations of total protein and phosphorus in the tubers, while a similar tendency occurred for potassium in tubers collected from the control plots.

2. Tubers of the cultivar Saturna accumulated more protein and less phosphorus and potassium than cv. Rywal, both after harvest and storage.

3. Concentration of protein in tubers of both cultivars (mean for the objects) was significantly lower after 3 and 6 month of storage at 8˚C in relation to the values recorded after harvest, while the contents of phosphorus and potassium increased significantly during storage.

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EFFECTS OF BORON FERTILIZATION OF SPRING CEREALS DEPENDING ON APPLICATION METHODS*

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Abstract

The study is a comparison of effects produced by boron fertilization applied to spring barley and oats, grown on light soil, low in available boron, in relation to the application method (pre-sowing, top dressing, foliar applications) and rates of the element. The study was carried out as a three-year series of one-year, two-factor strict field trials (the split-plot design). It has been demonstrated that top dressing with boron applied to soil at the tillering stage as well as foliar fertilization during the stem elongation stage can significantly improve yields. Grain of both cereals from the control plots showed symptoms of insufficient boron nutrition, which were absent when boron fertilization had been applied. Pre-sowing fertilization, although not affecting the yields, improved the supply of grain with boron. Differences between the cereal species were found in terms of boron concentrations in vegetative organs of the cereals and in their response to higher availability of this nutrient. Compared to spring barley, oats was characterised by a much higher content of boron in vegetative parts and was more responsive to increased concentrations of boron in soil.

Key words: boron deficiency, soil or foliar application of boron, boron concentration, yields.

EFEKTYWNOŚĆ NAWOŻENIA ZBÓŻ JARYCH BOREM W ZALEŻNOŚCI OD SPOSOBU APLIKACJI

Abstrakt

W badaniach porównywano efekty nawożenia borem jęczmienia jarego i owsa, uprawianych na glebie lekkiej o niskiej zawartości boru przyswajalnego, w zależności od sposo-

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bu stosowania (przedsiewnie, doglebowo pogłównie, dolistnie) oraz dawki. Badania przeprowadzono w jednorocznych dwuczynnikowych doświadczeniach polowych ścisłych (metodą split-plot), powtarzanych przez trzy lata. Wykazano istotne działanie plonotwórcze nawożenia doglebowego pogłównego borem, stosowanego w stadium krzewienia oraz dolistnego w stadium strzelania w źdźbło. Ziarno obu zbóż z obiektów kontrolnych wykazywało niedostateczne zaopatrzenie w ten składnik, które ulegało poprawie pod wpływem stosowania boru, w najwyższym stopniu – pogłównie doglebowo. Nawożenie przedsiewne nie wpływało na poziom plonowania, poprawiało jednak zaopatrzenie ziarna w badany mikroelement. Stwierdzono różnice międzygatunkowe w zawartości boru w częściach wegetatywnych zbóż oraz wrażliwość na zwiększoną dostępność tego składnika. Zawartość boru w częściach wskaźnikowych owsa była istotnie wyższa w porównaniu z jęczmieniem jarym i zwiększała się w większym stopniu wraz ze wzrostem tego składnika w glebie.

Słowa kluczowe: deficyt boru, nawożenie doglebowe lub dolistne borem, zawartość boru, plony.

INTRODUCTION

In the past, the recommendations on fertilization of older generations of cereals contained an assumption that boron fertilization was useless and sometimes even harmful to these crops. The concept was derived from such traits of cereal plants as low nutritional requirements for boron and a narrow gap between the optimum and excessive amounts of this microelement in plant tissues. Another important aspect was that cereal shoots were very sensitive to increased concentrations of boron in soil (TAHTINEN 1970). Cereals grown after a forecrop of root crops fertilized with boron (e.g. beetroot) responded negatively to increased boron concentrations in soil by producing lower yields. Under the agronomic technologies currently used in cereal crop cultivation, new, high-yielding generation cultivars of cereal crops, characterised by high nutritional demands, often struggle with insufficient supply of boron. Several factors can be involved, such as the widespread deficit of boron in soils, lack of manure, use of low-ballast fertilizers, frequent spells of dry weather in spring, simplified tillage and crop rotation systems (monocultures) (BELL 2000, HUANG et al. 2000). Another independent albeit important reason why boron fertilization can be a recommended procedure is the increasing nutritional demands (Biała Księga 2000). The results of several research projects completed in the USA suggest that boron plays an important role in the metabolism of higher organisms, which casts a new light on the value of this element in diets for mammals (NIELSEN 1996). Thus, good boron supply of cereals, staple foodstuff and fodder crops, acquires a new meaning.

The objective of the present study has been to evaluate the effect of boron fertilization applied to highly productive cultivars of spring barley and oats, observed in a strict field experiment set up on soil low in available boron. Three fertilization methods were assessed: pre-sowing, top-dressing and foliar.

METHODS

The study was carried out over the years 2001-2003 as a three-year series of one-year, two-factor strict field trials (the split-plot design) with spring barley and oats. The trials were established on boron-deficient soils (according to a 1 mol $HCl \cdot dm^{-3}$ test) at IUNG-PIB experimental stations. The experiment involving spring barley (cultivar Brenda) was carried out on a light (heavy loamy sand), slightly acidic soil, with a high content of phosphorus, potassium and magnesium, low concentrations of boron (0.70-1.30 mg $B \cdot kg^{-1}$) molybdenum and manganese, moderate supply of copper and very high level of zinc. The soils selected for the trials on oats (cultivar Borowiak) also belonged to light, acidic soils (light loamy sand) and contained high concentrations of phosphorus, potassium, magnesium and zinc, average content of copper, iron and manganese and low amounts of boron (0.60-0.80 mg $B \cdot kg^{-1}$) and molybdenum (Zalecenia nawozowe 1990).

The design of the experiment:

Factor I (boron application methods, A = 3): A_1 – pre-sowing, H_3BO_3 in the solid form (10-15 days before sowing), A_2 – top-dressing fertilization, H_3BO_3 in the solid form applied to soil at the tillering stage, A_3 – foliar, aqueous solution of H_3BO_3 applied at the stem elongation stage. Factor II – rates of boron, B = 5, (concentrations of H_3BO_3 solutions for foliar application in brackets): B_1 – control treatment - without boron fertilization, B_2 – 0.6 kg $B \cdot ha^{-1}$ (0.1% = 0.085 kg $B \cdot ha^{-1}$), B_3 – 1.2 kg $B \cdot ha^{-1}$ (0.2% = 0.170 kg $B \cdot ha^{-1}$), B_4 – 1.8 kg $B \cdot ha^{-1}$ (0.3% = 0.255 kg $B \cdot ha^{-1}$), B_5 – 2.4 kg $B \cdot ha^{-1}$ (0.4% = 0.340 kg $B \cdot ha^{-1}$). The experimental plots measured 10.0 m x 3.0 m when establishing the trials, and 10.0 x 2.5 m for the harvest. The cereals were grown according to the principles of rational agronomy, taking into account the soil and climatic conditions, stand, forecrop, soil fertility, etc. The weather conditions did not much differed over subsequent research years. Winter throughout the three years showed mild temperatures, higher that the multi-year norms, at lower precipitation. In 2001 March and July recorded heavier precipitation (about 35 and 70 mm above the multi-year mean, respectively). The two successive years kept mostly near-normal temperatures and precipitation with more considerable water deficits in June 2003 or in July 2002. Full chemical control of the experimental plots was performed. Grain yields were measured. For chemical analyses, the following were sampled: aerial parts of plants at the early heading stage (barley) or the early booting stage (oats) – the indicator parts according to BERGMANN & NEUBERT (1976), grain and straw at the harvest and soil from the arable layer (0-20 cm deep) after the termination of the trials. Chemical analyses of soil and plants were performed with the methods commonly used in agrochemical stations.The plant material after wet mineralization was determined to define the content of nitrogen with Kjeldahl method, phosphorus with the vanadium-molybdenum method, potassium and calcium – with the flame photometry and magnesium – with the AAS method. The content of microelements in dry mineralized plants was determined with the AAS method (Cu, Mn and Zn) except for colorimetrically determined boron – the method with curcumin and molybdenum – the thiocyanate method (Metody badań 1980). pH of soil in 1 mole KCl·dm^{-3}, granulometric composition of soil following Casagrande modified by Prószyński. The content of available forms of phosphorus and potassium in soil were determined with Egner and Riehm method, magnesium following Schachtschabel (Metody badań 1980). To determine the microelements (B, Cu, Mn, Mo, Zn), the soil was extracted with the so-called common extractant (1 mole HCl·dm^{-3}) following the instructions developed by IUNG (Gembarzewski, Korzeniowska 1990). Cu, Mn and Zn were determined with the AAS method, boron and molybdenum – calorimetrically. The results of the soil analyses were estimated with the applicable threshold values (Zalecenia Nawozowe 1990).

The results underwent statistical processing with analysis of variance, correlation and multiple regression with Statgraphics package and AWAR program (Filipiak, Wilkos 1995, Filipiak, Wilkos 1998). The significance of cross-object differences in variance analysis were evaluated with Tukey's test (a Ł 0.05).

RESULTS AND DISCUSSION

Similar tendencies in the response of grain yields of both cereals to boron fertilization were observed depending on the method of boron application and its rate. As a result of this interaction, statistically the highest yields were obtained in the sub-blocks which had received base top dressing fertilization and foliar fertilization, with the latter treatment method producing substantially better results, especially with respect to oats (Table 1). The fact that the cereals did not respond to pre-sowing boron application with improved grain yields can be attributed to the negative effect of boron on germinating cereal plants (Tahtinen 1970). Another factor which comes to play is the time lapse between the introduction of the fertilizer to soil and the moment when the plants' demand for this nutrient was the highest. In contrast, boron applied in a top-dressing treatment during the tillering stage was a good source of this element for the crops, which was reflected by the positive response to boron fertilization with both improved grain yields and higher boron concentration in grain. The highest rates of boron applied with this method caused non-significant decrease in yields, which confirmed the assumption that cereals are responsive to elevated availability of boron (Nable et al. 1997). Boron used for foliar treatments applied during the critical stage of the crop development proved to be most effective in stimulating higher yields. As the data set in table 1 indicate, this method of boron appli-

Table 1

Response of spring cereal grain yield to boron fertilization (three-year average)

Treatment			Spring barley	Oats	Mean
			Mg·ha^{-1}		cereal units·ha^{-1}
Fertilization	pre-sowing	A_1B_1	4.19	4.58	43.85
		A_1B_2	4.17	4.72	44.45
		A_1B3	4.21	4.70	44.55
		A_1B_4	4.20	4.65	44.25
		A_1B_5	4.22	4.49	43.55
	top-dressing	A_2B_1	4.17	4.58	43.75
		A_2B_2	4.38	4.74	45.60
		A_2B_3	4.40	5.12	47.60
		A_2B_4	4.37	5.15	47.60
		A_2B_5	4.35	4.94	46.45
	foliar	A_3B_1	4.16	4.57	43.65
		A_3B_2	4.50	5.21	48.55
		A_3B_3	4.50	5.22	48.60
		A_3B_4	4.40	5.26	48.30
		A_3B_5	4.45	5.32	48.85
Average		A_1	4.20	4.63	44.13
		A_2	4.33	4.91	46.20
		A_3	4.40	5.12	47.59
LSD $\alpha \leq 0.05$			0.19	0.23	2.08
Average		B_1	4.17	4.58	43.75
		B_2	4.35	4.89	46.20
		B_3	4.37	5.01	46.92
		B_4	4.32	5.02	46.72
		B_5	4.34	4.92	46.28
LSD $\alpha \leq 0.05$			n.s.*	n.s.	n.s.
LSD $\alpha \leq 0.05$		II/I	0.20	0.33	2.62
		I/II	0.18	0.39	2.77

* n.s. – non-significant differences

cation raised an average yield of cereal units within the range of 4.65-5.20 per ha. For oats, the three-year average grain yield increment ranged from 14.0 to 16.4% versus the control.

The initial boron concentration (in B1 objects without boron fertilization) in the indicator plant parts of both cereal crops was within the optimum range as established by BERGMANN & NEUBERT (1976). Nonetheless, it rose significantly in response to the fertilization. The highest increase in the boron levels in plant tissues occurred under the highest rate of boron, 2.4 kg B·ha^{-1}, applied to soil as a top-dressing treatment. The foliar application of boron to oats proved only slightly less effective. Both absolute values of boron concentrations in vegetative parts and increased boron levels caused by the fertilization were higher in the case of oats, which results mainly from the genetic traits of the two cereal species (RERKASEM, JAMOD 1997). For both cereals, the pre-sowing boron fertilization did not affect the yields but increased the concentration of boron in the indicator parts in a way that was similar to the effects produced by the top-dressing fertilization, which in turn caused an evident stimulating effect on the grain yields (Tables 1 and 2).

Boron supply of cereal grains showed somewhat different tendencies. By referring to the analogous data available from literature, it was discovered that the initial boron concentration in grains of both cereal crops was lower than the norm, but the boron fertilization treatments successfully improved the supply of this element, with the best results obtained when top-dressing applications were used. This finding is confirmed by the correlations: B soil/B barley grain $r = 0.77$; B soil/B oats grain $r = 0.82$; $\alpha = 0.01$. In the case of foliar application, boron concentration levels similar to the comparative data were obtained only when the highest boron rates had been applied. Top-dressing of oats with the highest boron rates proved to be less effective, both in terms of the resulting grain yield mass and the concentration of boron in grain (Tables 1 and 2). The results suggesting high effectiveness of boron top-dressing soil treatments in improving boron concentration in cereal grains are supported by earlier studies (RERKASEM et al. 1997, HUANG et al. 2001, WRÓBEL, SIENKIEWICZ-CHOLEWA 2004). Although foliar application is relatively less expensive, this method cannot compete with the classical soil fertilization, which guarantees ionic balance in soil and continuous dynamics in the uptake of this nutrient, which in turn ensures proper supply of grains with boron. This is also a result of the fact that boron is not reutilized in plants.

The next stage in the evaluation of the effect of a given boron fertilization treatment was the computation of quantitative relations between the content of boron and calcium in tissues of the index parts of barley and oats. As the two elements are antagonistic to each other, the ratio of their quantities in new plants is better at establishing the boron supply than the absolute concentration of boron in plants (SIMOJOKI 1991). The Ca:B values were narrower as the rates of boron increased, which proved that the boron supply of plants improved. The highest doses of boron depressed the mean Ca:B ratios for both cereal indicator parts (Table 2).

The results of the chemical analysis of soil samples after the termination of the trials showed that top-dressing application of boron significantly rose the content of its forms soluble in 1 mol HCl·dm^{-3}, considered to be available forms (GEMBARZEWSKI, KORZENIOWSKA 1990). In both series of the tri-

Table 2

Changes of B concentration and Ca:B quantitative ratio in cereals under boron fertilization (average effect)

Treatment			Barley		Oats		Barley	Oats
			indicator parts	grain	indicator parts	grain	Ca:B (indicator parts)	
			mg B · kg^{-1} of dry matter					
Fertilization	pre-sowing	A_1B_1	8.4	1.2	17.8	1.2	157	475
		A_1B_2	13.3	1.7	29.9	1.2	124	220
		A_1B3	15.4	1.8	31.3	1.3	97	172
		A_1B_4	15.1	1.8	36.3	1.4	107	153
		A_1B_5	18.3	1.7	45.5	1.3	82	120
	top-dressing	A_2B_1	8.2	1.1	18.2	1.2	160	436
		A_2B_2	9.3	1.2	40.3	1.3	133	128
		A_2B_3	13.2	1.8	49.1	1.4	108	113
		A_2B_4	15.7	1.8	50.4	1.8	101	93
		A_2B_5	19.0	2.3	52.1	1.7	77	81
	foliar	A_3B_1	7.8	0.8	17.9	1.1	168	481
		A_3B_2	8.2	1.0	29.2	1.1	154	315
		A_3B_3	8.1	1.0	35.3	1.3	157	237
		A_3B_4	8.9	1.1	38.4	1.3	144	205
		A_3B_5	9.1	1.3	44.0	1.5	146	188
Average		A_1	14.1	1.6	32.2	1.3	113	228
		A_2	13.1	1.6	42.0	1.5	114	202
		A_3	8.4	1.0	33.0	1.3	165	296
LSD $\alpha \leq 0.05$			3.89	0.42	n.s.*	n.s.	33.94	n.s.
Average		B_1	8.1	1.0	18.0	1.2	161.7	464
		B_2	10.3	1.3	33.1	1.2	137.0	221
		B_3	12.2	1.5	38.6	1.3	91.3	174
		B_4	13.2	1.6	41.7	1.5	117.3	150
		B_5	15.5	1.8	47.2	1.5	101.7	130
LSD $\alpha \leq 0.05$			4.74	0.44	19.33	0.17	34.40	139.94
LSD $\alpha \leq 0.05$		II/I	4.33	0.58	17.51	0.31	29.21	155.09
		I/II	4.09	0.54	n.s.	n.s.	23.16	n.s.
Reference levels								
			5-12**	1.34***	15 - 20**	1.44*-**		

* n.s. – non-significant differences
**after Bergmann and Neubert (1976), ***after Fotyma and Mercik (1995)

als, with barley and with oats, this meant that the available boron concentration in soil changed from class III (low) to II (moderate) (Zalecenia nawozowe 1990), Table 3.

By performing step-wise the multiple regression calculations, the effect of boron on higher grain yields was demonstrated in the form of functions. Assuming that grain yield was a dependent variable and the content of nutrients in plants and soil were independent variables, the following equations were obtained:

$$y_{barley} = 0.5009 + 0.2029\ B_{straw} + 0.0473\ P_{soil} + 0.0777\ K_{soil} \qquad R^2 = 0.871;\ \alpha \leq 0.05$$

$$y_{oats} = 0.8718 + 0.0412\ B_{straw} + 0.0842\ Mn_{grain} + 0.4536\ K_{i.p.} \qquad R^2 = 0.73;\ \alpha \leq 0.05$$

where:

- y – grain yield, in t per ha;
- B_{straw} – content of boron in straw of cereal crops, in mg $B \cdot kg^{-1}$ d.m.;
- Mn_{grain} – content of manganese in oats grain in mg $Mn \cdot kg^{-1}$ d.m.
- $K_{i.p.}$ – content of potassium in the indicator parts of oats plants, in mg $K \cdot kg^{-1}$ d.m.;
- P_{soil} K_{soil} – content of available forms of phosphorus and potassium in soil, in $mg \cdot kg^{-1}$ d.m. of soil.

High determination coefficients suggest that the above equations describe large part of the analysed variability of yields, which are significantly affected by a series of factors including the supply of vegetative plant parts (straw) with boron.

The results reported in this paper imply that the issue of boron fertilization of spring cereal crops is a complex one. The effectiveness of foliar application of boron in raising grain yield volumes does not coincide with the situation in which an appropriate level of this nutrient is guaranteed to occur in cereal grains. Plants grown on soils which are low in available boron will have an optimum amount of this element, and then boron fertilization causes substantial increments in the content of boron in the vegetative parts of the plants and in the grain. Certain differences between the two cereal species seem to occur, both in terms of their nutritional demands and sensitivities of the highest rates of boron tested in our study. Thus, it seems worth continuing the present research. The next steps should consist of verification of the criteria applied for assessment of the supply of plants with boron. The ranges of optimum supply suggested by Bergmann and Neubert (1976) concerned older generation of cereals and could be an inadequate reference for contemporary, high-yielding genotypes. Lack of proper criteria makes it impossible to assess precisely how cereal grain is supplied with boron. And such an evaluation will become a necessity under the new EU regulations which follow the principle ‘from field to table’. This

Table 3

Available boron content in soil after harvest (three-year average)

Treatment			mg B · kg^{-1}		
			spring barley	oats	average
Fertilization	pre-sowing	A_1B_1	0.88	0.50	0.69
		A_1B_2	1.21	0.33	0.77
		A_1B3	1.18	0.37	0.78
		A_1B_4	1.18	0.72	0.95
		A_1B_5	1.28	0.82	1.05
	top-dressing	A_2B_1	0.86	0.50	0.68
		A_2B_2	1.21	0.54	0.88
		A_2B_3	1.28	0.60	0.94
		A_2B_4	1.41	0.82	1.12
		A_2B_5	1.44	1.12	1.28
	foliar	A_3B_1	0.90	0.52	0.71
		A_3B_2	0.89	0.51	0.70
		A_3B_3	0.93	0.56	0.75
		A_3B_4	1.00	0.50	0.75
		A_3B_5	1.08	0.49	0.79
Average		A_1	1.15	0.55	0.85
		A_2	1.24	0.72	0.98
		A_3	0.96	0.52	0.74
LSD $\alpha \leq 0.05$			0.11	0.13	0.12
Average		B_1	0.88	0.51	0.69
		B_2	1.10	0.46	0.78
		B_3	1.13	0.51	0.82
		B_4	1.20	0.68	0.94
		B_5	1.27	0.81	1.04
LSD $\alpha \leq 0.05$			0.25	0.20	0.23
LSD $\alpha \leq 0.05$		II/I	0.22	0.19	0.21
		I/II	0.28	0.25	0.26

* n.s. – non-significant differences

principle sets forth certain requirements on the chemical composition of foodstuffs, particularly such important agricultural products as grains of cereal plants (Biała Księga 2000). The redefined nutritional recommendations regarding boron are a consequence of the latest global studies which clearly underline the important functions this element plays in the metabolism of calcium and fluorine in bodies of mammals (NIELSEN 1996, NIELSEN 1997). The suggested future studies should focus on nutritional demands of species and cultivars of various crops as well as their response to boron fertilization. The results of the studies conducted until now point to a disturbing fact that soil fertilization with boron is almost a non-existent procedure whereas sporadic fulfilment of plants' nutritional demands via foliar application of multi-component preparations can lead to producing grain that is insufficiently supplied with this important microelement, and such grain is of inferior biological and consumption quality.

CONCLUSIONS

1. Boron fertilization, and particularly soil top-dressing and foliar application of this element, caused significant increase in the grain yields produced by spring barley and oats, grown on soil low in available boron. Presowing fertilization, while not affecting the grain yields, improved the grain supply with boron.

2. Compared to spring barley, oats was characterised by a much higher content of boron in vegetative parts and was more responsive to increased concentrations of boron in soil.

3. Grain of both cereal crops grown under boron deficit in soil contained less than average amounts of this nutrient. Boron fertilization improved boron concentrations in grains. The best results in improving the supply of grain with boron were obtained under top-dressing soil application of this nutrient.

4. The results obtained in the present study suggest that grains produced on soils low in available boron by high yielding cultivars of spring barley and oats, which have high nutritional demands, might contain insufficient amounts of this element even if foliar fertilization had been applied, such as some of popular multi-component micronutrient preparations.

5. Owing to the complex nature of cereal fertilization with boron as well as growing nutritional demands of consumers, studies focusing on this question need to be continued.

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RESPONSE OF SPRING WHEAT TO FOLIAR FERTILIZATION WITH BORON UNDER REDUCED BORON AVAILABILITY*

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Abstract

The study focused on the effects of foliar fertilization with boron applied to spring wheat grown on sandy soil, low in available boron, under the conditions of simulated drought stress and the soil pH modified by liming. The study involved pot trials set up in a greenhouse. Wagner's pots, each containing 6 kg of light soil, served as experimental units. It was demonstrated that foliar application of boron was effective in mollifying the unfavourable wheat growth and nutrients uptake conditions (drought and soil reaction change). The fertilization alleviated the results of the limited availability of boron, significantly increasing the grain and straw yield mass and enriching the yields with boron. The highest rates of boron used for foliar application (7 and 9 cm^3 0.3% $H_3BO_3 \cdot pot^{-1}$) raised the concentration of this element in wheat grain up to a level comparable to the reference data.

Key words: drought, soil liming, boron fertilization, B content, yields.

REAKCJA PSZENICY JAREJ NA NAWOŻENIE DOLISTNE BOREM W WARUNKACH OGRANICZONEJ DOSTĘPNOŚCI TEGO SKŁADNIKA

Abstrakt

W badaniach określano efekty nawożenia dolistnego borem pszenicy jarej uprawianej na glebie lekkiej o niskiej zawartości boru dostępnego, w warunkach symulacyjnego stresu suszy i zmiany odczynu gleby wskutek wapnowania. Badania przeprowadzono w doświad-

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czeniach wazonowych, w hali wegetacyjnej. Jednostkę doświadczalną stanowiły wazony Wagnera mieszczące 6 kg gleby lekkiej. Wykazano skuteczność dolistnej aplikacji boru w łagodzeniu niesprzyjających warunków rozwoju pszenicy i pobierania składników (susza i zmiana odczynu gleby). Zastosowane nawożenie łagodziło skutki ograniczonej dostępności składnika, zwiększając istotnie masę plonów ziarna i słomy pszenicy jarej oraz wzbogacając je w bor. Najwyższe z zastosowanych w dolistnej aplikacji dawki boru (7 i 9 cm^3 0,3% $H_3BO_3 \cdot wazon^{-1}$) zwiększały zawartość tego składnika w ziarnie pszenicy do poziomu zbliżonego do porównawczego.

Słowa kluczowe: susza, wapnowanie gleby, nawożenie borem, zawartość B, plony.

INTRODUCTION

Although cereal crops, like other grasses, are less dependent on boron than dicotyledonous plants, it would be erroneous to assume that they did not need to be fertilized with this microelement. Among all major microelements, boron is the one that is most often found deficient in soil, which in turn leads to a deficit of this element in cereal crops (GOLDBACH 1997, GEMBARZEWSKI 2000). Positive response of grain plants to boron fertilization has been demonstrated in strict field experiments (WRÓBEL 2004, WRÓBEL 2006). Moreover, certain findings in physiology have abolished the notion that boron plays no role in the physiology of mammals (NIELSEN 1996). As a result, more attention is now being paid to proper boron nourishment of grain crops, one of the staple food and fodder plants in European agriculture (YAU 2000, BLEVINS, LUKASEVSKI 1998, WRÓBEL 2004, WRÓBEL 2006). Boron is taken up by plants mainly with the water transpiration stream, thus its availability for plants is worse during periods of drought, especially if the soil is low in this element. Elevated soil pH (e.g. after liming) has a similar effect on boron availability. These two factors can considerably inhibit the growth of plants, especially if they occur during critical plant growth phases.

The aim of the study has been to give scientific assessment of the effects of foliar fertilisation with boron applied to spring wheat grown under the deficit of boron in soil, which was subjected to simulated drought during a critical plant growth phase, in relationship with the soil reaction.

MATERIAL AND METHODS

The study involved pot trials set up in a greenhouse at the IUNG-PIB Experimental Station in Jelcz-Laskowice. Wagner's pots, each containing 6 kg of light soil, served as experimental units. The soil used for the trials was loamy sand, slightly acidic, rich in P, K and Zn, low in available B (0.9-1.1 mg $B \cdot kg^{-1}$ of dry soil according to the test with 1 mol $HCl \cdot dm^{-3}$)

and moderately rich in Mg, Cu, Mn and Mo (Zalecenia nawozowe 1990). A two-factor experiment with 5 replications was carried out for three years, according to the following design: first-order factor (A= 4) was boron availability: A1 – natural soil (pH = 5.7), during the whole growing season the soil moisture in the pot was maintained at 60% of the maximum water saturation (MWS); A2 – soil as above, limed with calcium carbonate according to single hydrolytic acidity (1 Hh), soil moisture at 60% MWS; A3 – natural soil, with the moisture content depressed to 40% MWS for 14 days since growth stage 61 on the Zadoks scale (onset of inflorescence emergence) (Rozdój 1996); A4 – limed soil (according to 1 Hh) + soil moisture lowered to 40% MWS for 14 days. The second-order factor (B = 5) comprised boron rates in foliar fertilization treatments applied during the tillering phase (0.3% H_3BO_3 solution per pot): B1 – control treatment (without boron fertilization), B2 = 3 cm^3, B3 = 5 cm^3, B4 = 7 cm^3, B5 = 9 cm^3. The desirable moisture of soil was established on the basis of determined maximum water saturation value. Soil moisture in pots was maintained by watering with deionizated water. The adequate amount of water for each pot individually was stated by weight.

Basic fertilization (in $g \cdot kg^{-1}$ soil) was: 0.2 N, 0.1 P, 0.2 K, 0.01 Mg, + microelements (in $mg \cdot kg^{-1}$ soil): 0.750 Cu, 3.50 Mn, 0.242 Mo, 2.50 Zn. Wheat (cultivar Ismena) was harvested at the full maturity phase. Lengths of growing seasons in the successive years of experiment were 91, 93 and 88 days. Grain and straw yields were determined.

Chemical analyses of soil (before experiment) and plants (grain and straw collected after the trials had been terminated) were performed with the methods commonly used in agrochemical stations. pH of soil in 1 mole $KCl \cdot dm^{-3}$, granulometric composition of soil following Casagrande modified by Prószyński. The content of available forms of phosphorus and potassium in soil were determined with the Egner and Riehm method, magnesium following Schachtschabel (Metody badań 1980). To determine the microelements (B, Cu, Mn, Mo, Zn), the soil was extracted with the so called common extractant (1 mole $HCl \cdot dm^{-3}$) following the instructions developed by IUNG (Gembarzewski, Korzeniowska 1990). Cu, Mn and Zn were determined with the AAS method, boron and molybdenum – colorimetrically. The results of the soil analyses were estimated with the applicable threshold values (Zalecenia Nawozowe 1990). The plant material after wet mineralization was determined to define the content of calcium – with the flame photometry. The content of boron in dry mineralized plants was determined colorimeterically – using the method with curcumin (Metody badań 1980).

This paper is a synthetic presentation of the results from the three years, processed statistically. The research result analysis involved variance and correlation analyses performed with AWAR and Statgraphics programs (Filipiak, Wilkos 1995). The significance of cross-object differences in variance analysis were evaluated with the Tukey's test ($\alpha \leq 0.01$).

RESULTS

The soil, initially slightly acidic in reaction (pH_{KCl} 5.7), was limed in sub-blocks A2 and A4 using $CaCO_3$ in a rate calculated according to 1 Hh, after which it was made more alkaline, reaching pH_{KCl} 6.3-6.6. In sub-block A2, this did not change significantly any of the yield components tested (plant height H, ear weight EW, number of grains per ear GE). However, under these conditions, foliar application of boron improved the analysed yield parameters more effectively than in the trials with natural soil (A1), thus the highest values of the above parameters were obtained in the fertilisation variants A2B4 and A2B5. The simulated drought stress in sub-bock A3 and, even more evidently, in limed soil (A4) significantly inhibited the development of spring wheat, which was reflected by statistically significant declines in the analysed yield components. In the object A4B1 the height of plants (H) was 27.6%, ear weight (EW) – 22.2% and number of grains per ear (GE) – 30.6% lower than in the initial object A1B1 (Table 1).

Higher rates of boron significantly alleviated this unwanted effect, although when compared to the sub-blocks receiving optimum water supply during the whole growing season (A1 and A2), the differences in the yield components often remained statistically significant. The analysed components of yield produced by spring wheat showed rather close correlation, including H/EW ($r = 0.708$; $\alpha = 0.01$); H/GE ($r = 0.639$; $\alpha = 0.01$) and EW/GE ($r = 0.733$; $\alpha = 0.01$). They were also closely correlated with spring wheat grain yield, e.g. grain yield /EW ($r = 0.801$; $\alpha = 0.01$) and straw yield, e.g. straw yield/H (r = 0.648; $\alpha = 0.01$). Thus, modifications in the analysed yield components caused by the interaction between the experimental factors had a significant effect on the mass of yields obtained. The liming of soil with $CaCO_3$ in a rate established according to 1 Hh in sub-block A2 did not cause any significant changes in grain and straw yields compared to spring wheat grown on natural soil (A1). However, significantly depressed grain and straw yields produced by spring wheat were caused by water deficit during the flowering phase (sub-block A3), especially when the soil had been limed (sub-block A4). Foliar application of boron raised the yields significantly, both in the sub-blocks receiving optimum water supply and those which underwent water deficit and soil reaction modification (Table 2).

The grain obtained on boron-deficient soil, irrespective of water supply or soil reaction, was characterised by a low concentration of this element versus the comparison data. Foliar application of boron increased its content in grain, with the actual improvement dependent on the water supply and soil reaction modification. The most positive changes in the boron content occurred in the control sub-block (A1) and limed sub-block (A2), in which higher boron fertilisation rates resulted in the boron content in grain exceeding the reference data. The least positive results were obtained from sub-block A4 (drought stress + liming), in which the supply of boron for

Table 1

Effect of boron foliar application on some components of the spring wheat yield (three-year average)

Treatment	Plant height cm (H)	Weight of ear g (EW)	Weight of ear in g (EW)	Number of grain per ear (GE)
A1	B1	88.0	1.35	37.6
	B2	91.2	1.56	43.7
	B3	92.1	1.67	44.0
	B4	93.5	1.62	45.9
	B5	90.7	1.70	44.6
A2	B1	86.4	1.30	36.6
	B2	90.0	1.52	44.7
	B3	90.9	1.58	44.0
	B4	92.4	1.75	47.0
	B5	92.3	1.78	47.2
A3	B1	67.7	1.15	30.2
	B2	70.4	1.33	30.9
	B3	71.3	1.35	31.4
	B4	78.3	1.43	32.5
	B5	78.4	1.51	34.8
A4	B1	63.7	1.05	26.1
	B2	67.7	1.03	25.9
	B3	67.5	1.13	27.9
	B4	69.9	1.33	30.5
	B5	72.8	1.48	32.3
Average	A1	91.1	1.58	43.2
	A2	90.4	1.59	43.9
	A3	73.2	1.35	32.0
	A4	68.3	1.20	28.5
LSD $_{\alpha \leq 0.01}$		9.14	0.29	6.08
Average	B1	76.5	1.21	32.6
	B2	79.8	1.36	36.3
	B3	80.5	1.43	36.8
	B4	83.5	1.53	39.0
	B5	83.6	1.62	39.7
LSD $_{\alpha \leq 0.01}$		6.18	0.31	5.95
LSD $_{\alpha \leq 0.01}$	II/I	5.66	0.34	3.90
	I/II	7.85	0.28	4.49

Table 2

Yields of spring wheat grain and straw under boron treatment
(three-year average)

Treatment		Grain	Straw
		g · pot^{-1}	
A1	B1	27.1	33.9
	B2	30.0	34.6
	B3	30.5	36.6
	B4	31.0	36.7
	B5	31.4	34.2
A2	B1	27.0	34.1
	B2	28.8	36.0
	B3	30.9	36.5
	B4	32.0	37.3
	B5	30.3	35.9
A3	B1	24.9	30.3
	B2	25.5	32.5
	B3	26.9	34.0
	B4	28.1	35.6
	B5	29.2	35.9
A4	B1	23.8	29.5
	B2	24.4	30.7
	B3	25.6	32.2
	B4	27.3	34.2
	B5	28.3	34.5
Average	A1	30.0	35.2
	A2	29.8	36.0
	A3	26.9	33.7
	A4	25.9	32.2
LSD $_{\alpha \leq 0.01}$		2.58	2.87
Average	B1	25.7	32.0
	B2	27.2	33.5
	B3	28.5	34.8
	B4	29.6	36.0
	B5	29.8	35.1
LSD $_{\alpha \leq 0.01}$		2.57	2.66
LSD $_{\alpha \leq 0.01}$	II/I	3.55	2.75
	I/II	3.03	n.s.*

*n.s. – non-significant differences

Table 3

Content of boron and Ca:B quantitative ratio in the spring wheat grain and straw (three-year average)

Treatment		Grain mg · kg^{-1} dry matter		Ca:B	Straw mg · kg^{-1} dry matter		Ca:B
A1	B1	1.35	441	327	4.35	1720	395
	B2	1.47	464	316	4.97	1914	385
	B3	1.64	506	308	5.64	1963	348
	B4	1.74	496	285	5.74	1847	321
	B5	1.69	485	287	5.69	1809	318
A2	B1	1.00	388	388	3.91	1810	463
	B2	1.38	523	379	4.79	2060	430
	B3	1.45	536	370	4.88	2060	422
	B4	1.68	588	350	5.71	2146	376
	B5	1.67	576	345	5.85	2135	365
A3	B1	1.09	363	333	3.09	1225	396
	B2	1.08	352	326	3.27	1276	390
	B3	1.35	443	328	3.65	1340	367
	B4	1.48	458	310	4.21	1390	330
	B5	1.56	490	314	4.33	1380	319
A4	B1	1.00	392	392	2.97	1340	451
	B2	1.08	410	379	3.08	1340	435
	B3	1.15	443	385	3.22	1295	402
	B4	1.33	478	360	3.73	1436	385
	B5	1.37	485	354	3.88	1478	381
Average	A1	1.58	478	305	5.28	1851	353
	A2	1.44	522	366	5.03	2042	411
	A3	1.31	421	322	3.71	1322	360
	A4	1.19	442	374	3.38	1378	411
LSD $_{\alpha \leq 0.01}$		0.20	72.4	58.1	0.97	189.5	46.7
Average	B1	1.11	396	360	3.58	1524	426
	B2	1.25	437	350	4.03	1648	410
	B3	1.40	482	348	4.35	1665	385
	B4	1.56	505	326	4.85	1705	353
	B5	1.57	509	325	4.81	1701	346
LSD $_{\alpha \leq 0.01}$		0.22	77.0	33.1	0.69	176.3	38.9
LSD $_{\alpha \leq 0.01}$	II/I I/II	0.27 0.24	49.4 45.2	36.5 55.0	0.72 1.18	164.8 283.5	44.2 56.6
Reference B content after Fotyma and Mercik (1995)							
		1.52	800	-	3.25	2700	-

wheat grain did not reach the reference data (Table 3). The initial content of boron in wheat straw was high, surpassing the reference data. However, the straw of wheat plants grown under drought conditions was clearly inferior in the amounts of this microelement and satisfactory improvement was obtained only in the objects fertilised with the highest rates of boron (Table 3).

These observations are confirmed by the values of the Ca:B quantitative ratio in spring wheat grain and straw, which characterises plants' nutrition with boron more fully than the absolute content of boron in plant dry matter. Even when plants receive quite a good supply of boron, excessive amounts of calcium in plant tissues may create boron deficiency conditions (Gupta 1972, Kopeć, Michalec 2007). It is so because of antagonism between these two elements. Any narrowing of the Ca:B ratio suggests improved supply of plants with boron. The comparison of the Ca:B ratios from particular experimental objects proves the effectiveness of boron application as a means of improving plants' supply with boron. Changes in the Ca:B ratio occurring in the experimental objects depended mainly on changes in the content of boron in plant tissues, thus they were more evident in straw rather than in grain. Both in grain and in straw of spring wheat, the Ca:B quantitative ratio was broader in limed sub-blocks (A2 and A4), which was due to the increased supply of calcium. Under such conditions, the demand of wheat plants for boron also increased. As a result of foliar application of boron, the Ca:B ratio changed in a reversely proportional correlation to the volume of the boron rate applied (Table 3).

DISCUSSION

The interaction between the experimental factors (availability of boron x foliar fertilisation with boron) caused significant changes in the analysed yield structure of spring wheat. These changes affected the yields of grain and straw. Water deficit during the inflorescence phase (from growth stage 61 according to the Zadoks scale) may create a considerable threat to spring wheat yields (Mouhouche et al. 1998, Huang et al. 2000). One of the reason could be the deficit of boron in plants resulting from the fact that the uptake of this element by plants is largely limited under drought conditions (plants take up boron with the water transpiration stream) as well as the lack of reutilisation of boron in plants (Goldbach 1997, Blevins, Lukasewski 1998, Thellier et al. 2001). Another cause why the availability of boron was limited might have been increased soil pH. At $pH_{KCl} > 6.0$, boron occurs in soil solution as anions of boron hydrides $B(OH)_4^-$, which can easily be absorbed by loamy minerals, aluminium hydroxides and organic substances.

The physiological functions of boron in a plant, including the effect of this element on the development of generative parts, are decisive for the proper development and maturation of cereal grains (GOLDBACH 1997, HUANG et al. 2000, YAU 2000) thus by supplying additional amounts of boron in the form of foliar application during the critical growth phases can be perceived as a factor which mollifies the results of unfavourable boron deficits. Foliar fertilization with boron in the experiments leads to a statistically significant improvement of yield structure parameters and spring wheat yield volumes. This type of a relationship is further verified by relatively high coefficients of B in straw/plant height ($r = 0.667$; $\alpha = 0.01$), B in straw/weight of ear ($r = 0.511$; $\alpha = 0.01$), B in grain/grain yield ($r = 0.562$; $\alpha = 0.01$), B in straw/straw yield ($r = 0.622$; $\alpha = 0.01$).

The deficit of available boron in the soil used for our trials, made more severe due to the drought stress or when the soil reaction was more alkaline, may have implied that plants received insufficient amounts of boron as a nutrient. As one could have expected, the content of boron in plants from the control objects (B1 – without boron fertilization) was too low versus the data supplied by the references (FOTYMA, MERCIK 1995). The highest rates of boron applied for foliar treatments increased the content of boron in grains to a level comparable to the standard, in which case the fertilisation treatment played a double role: it increased the grain yield and improved its nutritional quality (Tables 2, 3). In sub-block A4 (periodic drought + soil liming), such a level of boron concentration was not achieved. Insufficient content of boron in wheat grain can raise some anxiety in terms of nutritional quality of grain for human consumption or animal fodder (BIAŁA KSIĘGA 2000). As some research seems to suggest, boron deficit in food or fodder can be responsible for health disorders in man and animals (NIELSEN 1997).

CONCLUSIONS

1. The deficit of boron available in soil had a negative effect on the spring wheat yield structure parameters and yield level as well as the concentration of boron in grain and straw. This correlation was stronger when boron availability was depressed (periodic drought, soil liming).

2. Foliar fertilization with boron has a favourable effect on the major wheat yield components, significantly improving grain and straw yields.

3. The highest rates of boron used for foliar application (7 and 9 ml 0.3% H_3BO_3/pot) raised the concentration of this component in wheat grain up to a level comparable to the reference data.

4. In the light of such positive effects of foliar fertilization with boron as presented in this paper, it can be stated that boron fertilisation is a treatment which can mollify results of less favourable conditions for boron uptake by plants.

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EFFECT OF COMPOST, BENTONITE AND CALCIUM OXIDE ON CONTENT OF SOME MACROELEMENTS IN PLANTS FROM SOIL CONTAMINATED BY PETROL AND DIESEL OIL*

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Abstract

The aim of the study was to determine how soil contamination with petrol and diesel oil affected content of some macroelements in spring oilseed rape (*Brassica napus* var. *oleifera*) and oat (*Avena sativa* L.) and to determine whether application of compost, bentonite or calcium oxide could reduce the impact of petroleum-derived products on the properties of the plants. The soil formed from sandy loam was polluted with the following amounts of petrol and diesel oil: 2.5, 5.0 and 10 $cm^3 \cdot kg^{-1}$ of soil. The results of the tests showed that contamination of soil with diesel oil at the amount between 2.5 and 10 $cm^3 \cdot kg^{-1}$ of soil disturbed the plants' chemical composition. Irrespective of the application of compost, bentonite or calcium oxide, the highest doses of petrol and especially diesel oil decreased the content of most macroelements in spring oilseed rape and, to a smaller degree, in oat. Enrichment of soil with compost, bentonite or calcium oxide modified the content of macroelements in plants, mainly that of sodium under the effect of bentonite. Significant correlations, mainly between the content of some macroelements in spring oilseed rape and oats versus plant yield and content of other elements in plants, as well as some properties of soil were observed.

Key words: petrol and diesel oil contamination, compost, bentonite, calcium oxide, spring oilseed rape, oat, macroelements content.

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WPŁYW KOMPOSTU, BENTONITU I TLENKU WAPNIA NA ZAWARTOŚĆ NIEKTÓRYCH MAKROSKŁADNIKÓW W ROŚLINACH Z GLEBY ZANIECZYSZCZONEJ BENZYNĄ I OLEJEM NAPĘDOWYM

Abstrakt

Celem badań było określenie, w jaki sposób zanieczyszczenie gleby benzyną i olejem napędowym wpływa na zawartość niektórych makroskładników w rzepaku jarym (*Brassica napus* var. *oleifera*) i owsie (*Avena sativa* L.) oraz czy stosowanie kompostu, bentonitu i tlenku wapnia mogłoby zmniejszyć oddziaływanie substancji ropopochodnych na badane cechy roślin. Doświadczenia przeprowadzono na glebie wytworzonej z piasku gliniastego zanieczyszczonej rosnącymi dawkami benzyny i oleju napędowego: 2,5, 5,0 i 10 $cm^3 \cdot kg^{-1}$ gleby. W wyniku badań wykazano, że zanieczyszczenie gleby olejem napędowym w dawkach od 2,5 do 10 $cm^3 \cdot kg^{-1}$ gleby modyfikuje skład chemiczny roślin. Najwyższe dawki benzyny i oleju napędowego, niezależnie od aplikacji kompostu, bentonitu i tlenku wapnia, zmniejszały zawartość większości makroskładników w rzepaku jarym, i w mniejszym stopniu w owsie. Wzbogacenie gleby w kompost, bentonit i tlenek wapnia modyfikowało zawartość makroskładników w roślinach, głównie sodu, po zastosowaniu bentonitu. Istotne korelacje stwierdzono głównie między zawartością niektórych makroskładników w rzepaku jarym i owsie oraz plonem roślin i zawartością innych składników w roślinach, a także niektórymi właściwościami gleby.

Słowa kluczowe: zanieczyszczenie benzyną i olejem napędowym, kompost, bentonit, tlenek wapnia, rzepak jary, owies, zawartość makroskładników.

INTRODUCTION

Petroleum-derived products, which are widespread the natural environment, contribute to soil degradation by deteriorating soil, air and water chemical properties (WANG, BARTHA 1990, IWANOW et al. 1994, SZTOMPKA 1999). Petroleum-derived products include aliphatic, oleic and naphthenic hydrocarbons (CHI, KRISHNAMURTHY 1995) and water-soluble aromatic hydrocarbons, such as benzene, toluene, xylene (KRAHL et al. 2002). Polycyclic aromatic hydrocarbons (PAH) and other aromatic hydrocarbons are hardly mobile and can have a long-term effect on soil, plants or ground waters (SPARROW, SPARROW 1988, RACINE 1993, WYSZKOWSKA et al. 2002a,b). Contaminants penetrating soil disturb its structure and modify its physicochemical and biological properties (SZTOMPKA 1999, BUDNY et al. 2002, CARAVACA, RODÁN 2003). Soil enzymatic and microbiological activities often respond to soil pollution with petroleum products (CARAVACA, RODÁN 2003, WYSZKOWSKA, WYSZKOWSKI 2006). Polycyclic aromatic hydrocarbons are toxic to soil organisms, plants (BUDNY et al. 2002, WYSZKOWSKA et al. 2002a,b, DELILLE et al. 2003) and people (KRAHL et al. 2002). These contaminants can show potential carcinogenic and mutagenic activity (KRAHL et al. 2002). **T**he content of available macro- and microelement forms in soil contaminated with petroleum-derived products (WYSZKOWSKI, ZIÓŁKOWSKA 2007) and the uptake by plants of macroelements changes so that the content of nutrients in particular plant organs fluctuates (WYSZ-

KOWSKI et al. 2004). It is, therefore, extremely important to restore soil contaminated with petroleum-derived products to its original state.

The aim of the study was to determine the effect of petrol and diesel oil soil contamination on the content of some macroelements in spring oilseed rape (*Brassica napus* var. *oleifera*) and oat (*Avena sativa* L.), and to verify whether soil amendment with compost, bentonite and calcium oxide could reduce the impact of petroleum-derived products on the properties of the plants.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the University of Warmia and Mazury in Olsztyn (Poland), in polyethylene pots (with 4 replications). Soil material used for the trials was taken from the arable humus soil horizon and, under natural conditions, it was proper Eutric Cambisols soil according to WRB (1998) formed from sandy loam (1.0-0.1 mm – 50%; 0.1-0.02 mm – 39%; <0.02 mm – 8%), characterised by following properties: pH in 1 M KCl dm^{-3} – 5.10; hydrolytic acidity (HA) – 30.8 mmol $(H^+)\cdot kg^{-1}$; exchangeable cation bases – Ca^{++}, Mg^{++}, K^+ and Na^+ (ECB) – 88.0 mmol(+)$\cdot kg^{-1}$; cation exchange capacity (CEC) – 118.8 mmol(+)$\cdot kg^{-1}$; base saturation (BS) – 74.1%; C_{org} content – 8.48 $g\cdot kg^{-1}$; content of available: phosphorus – 34.1 $mg\cdot kg^{-1}$; potassium – 75.2 $mg\cdot kg^{-1}$ and magnesium – 36.7 $mg\cdot kg^{-1}$. During the study, increasing doses of petrol and diesel oil were applied in the following amounts: 0; 2.5; 5 and 10 $cm^3\cdot kg^{-1}$ d.m. of soil. Afterwards, some objects were enriched with compost (3% of the soil mass), bentonite (2%) or calcium oxide (in a dose equal one full hydrolytic acidity – 1.47 g $Ca\cdot kg^{-1}$ of soil). Compost was prepared from leaves (44%), manure (33%) and peat (23%) composted for six months. The concentration of macroelements in these substances (in $g\cdot kg^{-1}$) was as follows: compost: P – 2.32, K – 1.33, Mg – 1.47, Ca – 15.86, Na – 0.12; bentonite: P – 0.47, K – 2.43, Mg – 5.03, Ca – 26.72, Na – 12.11; calcium oxide: P – 0.10, K – 0.77, Mg – 2.65, Ca – 347.99, Na – 0.07. Additionally, macro- and microelements were added to all pots, in the following amounts (in $mg\cdot kg^{-1}$ of soil): N – 150 $CO(NH_2)_2$; P – 30 (KH_2PO_4); K – 70 (KH_2PO_4 + KCl); Mg – 50 ($MgSO_4.7H_2O$); Mn – 5 ($MnCl_2.4H_2O$); Mo – 5 ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$); B – 0.33 ($H_3BO_3$). The mineral fertilizers, in the form of aqueous solution, as well as petroleum-derived substances, compost, bentonite and calcium oxide, wherever appropriate, were introduced to the soil once prior to sowing spring oilseed rape, by mixing the substances with the whole mass of soil per pot. The soil samples thus prepared (each 9.5 kg) were placed in pots, where their moisture level was brought up to 60% of capillary water capacity. Prior to mixing and placing in pots, the soil was passed through a sieve with a mesh size 1 cm^2 and mixed with mineral fertilizers and in some objects with diesel oil as well as compost, bentonite and CaO. Afterwards, Polish cultivar Mazowiecki spring oilseed rape (*Brassica napus* var. *oleifera*) was sown, which, after the harvest, was followed by Polish cv. Borowik oats (*Avena sativa* L.). After emergence, 8 plants of spring oilseed rape per pot and 15 plants of oats per pot were left to grow. Immediately after harvesting the main crop in the flowering stage (58 day of vegetation) and collecting plants samples for chemical analyses, oats as an aftercrop was sown. The harvest of oats was carried out in the panicle stage (52 day of vegetation), after which plant samples were collected. The experiment was conducted in four replications. During the experiment (110 days), the moisture of soil was maintained at the level of 60% capillary water capacity.

After the vegetation period, the aerial parts of plants were measured for each pot and the plant material was sampled for laboratory analyses. The samples were cut, dried and ground. Next, they were mineralised in 25 cm^3 of concentrated H_2SO_4 with the addition of 1 g of d.m of hydrogen peroxide as a catalyst. The mineralised samples were transferred into conical flasks, which were replenished with distilled water to the volume of 200 cm^3 and assayed for macroelement content. The plant material was analysed for phosphorus by colorimetry (CAVELL 1955). Potassium, calcium and sodium were determined with atomic emission spectroscopy (AES) (SZYSZKO 1982). Magnesium was assayed with atomic absorption spectroscopy (AAS) (SZYSZKO 1982). Prior to plant sowing, the following soil properties were determined: pH (exchangeable acidity) potentiometrically, using aqueous solution of KCl at the concentration of 1M KCl dm^{-3} (LITYŃSKI et al. 1976), hydrolytic acidity – exchangeable H^+ and Al^{+++} (HA) and exchangeable cation bases – Ca^{++}, Mg^{++}, K^+ and Na^+ (ECB) with Kappen method (LITYŃSKI et al. 1976), content of organic carbon (C_{org}) with Tiurin method, using potassium dichromate with diluted sulphuric acid (LITYŃSKI et al. 1976), the content of available phosphorus and potassium – by Egner-Riehm method (LITYŃSKI et al. 1976), the content of available magnesium – by Schachtschabel method (LITYŃSKI et al. 1976). Based on the hydrolytic acidity and exchangeable cation bases, the cation exchange capacity (CEC) and base saturation (BS) were calculated from the following formulas: CEC = ECB + HA, BS = $(ECB \cdot CEC^{-1}) \cdot 100$. The results were analysed statistically using three-factor ANOVA and two-factor analysis of variance with Statistica software (StatSoft Inc. 2005). Finally, based on the results, Pearson's simple correlation coefficients between the variables tested experimentally were calculated for all replications.

RESULTS AND DISCUSSION

Effect of petrol and diesel oil on content of some macroelements in plants

The present study has demonstrated that the actual effect of petrol and diesel oil on the content of the macroelements in plants depended on the degree of contamination, application of a neutralizing substance (compost, bentonite and calcium oxide) as well as on the species of a crop, which determined the duration of contamination impact (Tables 1-5, Figure 1). The correlations between doses of petroleum-derived products and content of macroelements in plants were stronger for spring oilseed rape (grown as the main crop) than for oats (the aftercrop). The correlations were stronger

Table 1

Effect of petrol and diesel oil contamination on phosphorus (P) content in aerial parts of plants, in ($g \cdot kg^{-1}$ d.m.)

Dose of Pet or DO ($cm^3 \cdot kg^{-1}$ of soil)	Contamination							
	petrol (Pet)				diesel oil (DO)			
	kind of substance neutralizing effect of Pet and DO							
	without additions	compost	bentonite	CaO	without additions	compost	bentonite	CaO
Spring oilseed rape (*Brassica napus* var. *oleifera*) – main crop								
0	3.15	4.14	3.87	4.25	3.15	4.14	3.87	4.25
2.5	4.66	4.59	4.71	4.69	4.05	4.04	3.72	3.36
5.0	6.18	5.52	5.02	4.55	3.87	4.08	4.17	3.94
10.0	4.90	4.05	5.27	4.17	3.14	5.16	5.96	4.10
r	0.560	-0.057	0.899**	-0.346	-0.212	0.854**	0.920**	0.127
LSD	*a* – 0.04 **, *b* – 0.06 **, *c* – 0.06 **, $a \cdot b$ – 0.08 **, $a \cdot c$ – 0.08 **, $b \cdot c$ – 0.12 **, $a \cdot b \cdot c$ – 0.16 **							
Oats (*Avena sativa* L.) – aftercrop								
0	1.42	1.51	1.69	1.49	1.42	1.51	1.69	1.49
2.5	1.42	1.62	1.49	1.55	1.33	1.52	1.88	1.52
5.0	1.31	1.50	1.42	1.45	1.67	1.71	1.79	1.36
10.0	1.24	1.39	1.50	1.33	1.45	1.47	1.88	1.49
r	-0.954**	-0.737**	-0.582	-0.872**	0.267	-0.086	0.668*	-0.150
LSD	*a* – 0.02**, *b* – 0.03 **, *c* – 0.03 **, $a \cdot$b – 0.04 **, $a \cdot c$ – 0.04 **, $b \cdot c$ – 0.05 **, $a \cdot b \cdot c$ – 0.07 **							

LSD for: *a* – petroleum substance, *b* – petroleum substance dose, *c* – neutralizing substance
**significant at *p*=0.01, *significant at *p*=0.05, *r* – correlation coefficient

Table 2

Effect of petrol and diesel oil contamination on potassium (K) content in above-ground parts of plants, in $(g \cdot kg^{-1}$ d.m.)

Dose of Pet or DO ($cm^3 \cdot kg^{-1}$ of soil)	Contamination							
	petrol (Pet)				diesel oil (DO)			
	kind of substance neutralizing effect of Pet and DO							
	without additions	compost	bentonite	CaO	without additions	compost	bentonite	CaO
Spring oilseed rape (*Brassica napus* var. *oleifera*) – main crop								
0	16.60	20.07	17.99	19.66	16.60	20.07	17.99	19.66
2.5	20.49	18.47	22.63	21.42	17.15	19.55	15.88	14.93
5.0	26.50	21.25	21.85	18.92	20.50	23.78	15.67	17.87
10.0	25.20	15.90	22.01	17.38	23.36	31.73	31.46	26.80
r	0.813**	-0.665*	0.603*	-0.772**	0.977**	0.956**	0.810**	0.741**
LSD	*a* – 0.32 *, *b* – 0.46 **, *c* – 0.46 **, $a \cdot b$ – 0.65 **, $a \cdot c$ – 0.65 **, $b \cdot c$ – 0.92 **, $a \cdot b \cdot c$ – 1.29 **							
Oats (*Avena sativa* L.) – aftercrop								
0	16.55	16.03	18.66	17.13	16.55	16.03	18.66	17.13
2.5	16.86	18.12	19.70	17.81	24.61	24.40	20.62	23.35
5.0	16.74	18.53	19.56	18.23	27.47	27.22	22.00	24.58
10.0	23.49	25.75	22.04	23.76	29.49	26.80	23.98	24.89
r	0.889**	0.964**	0.954**	0.939**	0.886**	0.778**	0.986**	0.787**
LSD	*a* – 0.38 **, *b* – 0.53 **, *c* – 0.53 **, $a \cdot b$ – 0.75 **, $a \cdot c$ – 0.75 **, $b \cdot c$ – 1.07 **, $a \cdot b \cdot c$ – 1.51 **							

LSD for: *a* – petroleum substance, *b* – petroleum substance dose, *c* – neutralizing substance
**significant at p=0.01, *significant at p=0.05, *r* – correlation coefficient

in the objects with petrol than in the variants with diesel oil. In the first series of experiments (without compost, bentonite or CaO), petrol stimulated the content of calcium (r=0.877) and magnesium (r=0.969) in aerial parts of spring oilseed rape (main crop). In the soil samples mixed with 10 cm^3 $petrol \cdot kg^{-1}$, calcium content in spring oilseed rape was 118% and magnesium content was 37% higher than in the non-contaminated objects. Identical effects were produced by 5 cm^3 $petrol \cdot kg^{-1}$ of soil on the content of phosphorus (r=0.560), potassium (r=0.813) and sodium (r=0.813). For these objects, the increase reached 96, 60 and 185%, respectively, compared to the control variant (without petrol). The highest dose of petrol (10 $cm^3 \cdot kg^{-1}$ of soil) resulted in a decrease in the content of phosphorus, potassium and sodium in spring oilseed rape. Diesel oil (without any organic substance or CaO added) stimulated only the potassium content (r=0.977) in the aerial

Table 3

Effect of petrol and diesel oil contamination on sodium (Na) content in aerial parts of plants ($g \cdot kg^{-1}$ d.m.)

Dose of Pet or DO ($cm^3 \cdot kg^{-1}$ of soil)	Contamination							
	petrol (Pet)				diesel oil (DO)			
	kind of substance neutralizing effect of Pet and DO							
	without additions	compost	bentonite	CaO	without additions	compost	bentonite	CaO
Spring oilseed rape (*Brassica napus* var. *oleifera*) – main crop								
0	1.14	1.47	6.01	1.62	1.14	1.47	6.01	1.62
2.5	2.01	1.28	10.38	1.47	2.11	2.53	6.25	1.23
5.0	3.25	2.51	10.39	1.56	1.22	1.40	5.78	0.91
10.0	2.98	2.51	12.43	2.06	1.15	0.79	13.63	0.70
r	0.813**	0.811**	0.885**	0.809**	-0.284	-0.633*	0.865**	-0.951**
LSD	*a* – 0.06 **, *b* – 0.09 **, *c* – 0.09 **, *a·b* – 0.12 **, *a· c* – 0.12 **, *b·c* – 0.18 **, *a·b·c* – 0.25 **							
Oats (*Avena sativa* L.) – aftercrop								
0	7.67	7.67	17.26	10.41	7.67	7.67	17.26	10.41
2.5	4.91	7.45	16.03	8.96	2.56	3.61	17.29	3.51
5.0	6.18	7.51	16.20	7.69	1.60	1.76	17.47	1.49
10.0	0.77	5.69	14.75	4.05	1.51	2.42	14.86	5.22
r	-0.910**	-0.907**	-0.946**	-0.997**	-0.769**	-0.755**	-0.843**	-0.446
LSD	a – 0.14 **, b – 0.20 **, c – 0.20 **, a·b – 0.28 **, a·c – 0.28 **, b· c – 0.39 **, a· b·c – 0.56 **							

LSD for: *a* – petroleum substance, *b* – petroleum substance dose, *c* – neutralizing substance
**significant at p=0.01, *significant at p=0.05, *r* – correlation coefficient

parts of spring oilseed rape, where the increase was 41%. Similar correlations were found for phosphorus (2.5 $cm^3 \cdot kg^{-1}$ of soil), sodium (2.5 $cm^3 \cdot kg^{-1}$ of soil), calcium (5 $cm^3 \cdot kg^{-1}$ of soil) and magnesium (5 $cm^3 \cdot kg^{-1}$ of soil). This effect was particularly strong, which was evidenced by the increase in calcium (105%) and magnesium (103%). The application of 10 cm^3 of diesel oil per 1 kg of soil caused considerable decrease in phosphorus, sodium, calcium and magnesium, particularly in aerial parts of spring oilseed rape.

The effect of petroleum-derived products on the content of macroelements in aerial parts of oats (aftercrop) was weaker (Tables 1-4). In the first (control) series, the content of phosphorus, sodium and magnesium in aerial parts of oats decreased by 13% (*r*=-0.954), 90% (*r*=-0.910) and 22% (*r*=-0.944), respectively, in objects with petrol The content of sodium, calcium and magnesium content decreased by nearly 80% (r=-0.769), 21% (r=-0.663) and 14%

Table 4

Effect of petrol and diesel oil contamination on calcium (Ca) content in above-ground parts of plants ($g \cdot kg^{-1}$ d.m.)

Dose of Pet or DO ($cm^3 \cdot kg^{-1}$ of soil)	Contamination							
	petrol (Pet)				diesel oil (DO)			
	kind of substance neutralizing effect of Pet and DO							
	without additions	compost	bentonite	CaO	without additions	compost	bentonite	CaO
Spring oilseed rape (*Brassica napus* var. *oleifera*) – main crop								
0	8.47	9.71	9.48	12.37	8.47	9.71	9.48	12.37
2.5	8.70	10.85	11.70	13.03	15.08	16.31	12.05	12.77
5.0	9.92	11.59	11.45	10.89	17.38	17.24	15.87	16.27
10.0	9.96	11.26	12.73	12.78	10.61	18.9	15.58	17.34
r	0.877**	0.739**	0.882**	0.012	0.100	0.853**	0.857**	0.930**
LSD	*a* – 0.23 **, *b* – 0.33 **, *c* – 0.33 **, *a·b* – 0.46 **, *a·c* – 0.46 **, *b·c* – 0.65 **, *a·b·c* – 0.92 **							
Oats (*Avena sativa* L.) – aftercrop								
0	5.54	4.69	4.74	6.22	5.54	4.69	4.74	6.22
2.5	5.48	5.16	4.16	6.87	5.34	5.65	4.32	6.43
5.0	5.73	5.85	4.14	6.22	6.01	6.63	4.09	6.51
10.0	5.65	4.69	3.85	6.69	4.40	5.14	3.87	6.21
r	0.594	-0.022	-0.899**	0.336	-0.663*	0.182	-0.947**	-0.139
LSD	*a* – n.s., *b* – 0.24 **, *c* – 0.24 **, *a·b* – 0.34 *, *a·c* – 0.34 **, *b·c* – 0.68 **, *a·b·c* – n.s.							

LSD for: *a* – petroleum substance, *b* – petroleum substance dose, *c* – neutralizing substance
**significant at p=0.01, *significant at p=0.05, *r* – correlation coefficient

(r=-0.577) in oats cultivated in soil contaminated with 10 cm^3 of diesel oil per 1 kg of soil. The highest petrol and diesel oil doses increased only potassium in oat, whereas the middle diesel oil dose (5 $cm^3 \cdot kg^{-1}$ of soil) depressed only the content of phosphorus. Further increases in petrol and diesel oil doses had a negative effect on the content of phosphorus and potassium in oats.

The study presented in this paper has revealed some strong and significant correlations between the content of macroelements in spring oilseed rape and oats versus plant yield and content of other elements in plants, as well as some properties of soil (Table 6). Such relationships are confirmed especially by correlation coefficients calculated between the content of potassium, sodium and calcium and other elements in plants and properties of soil.

Table 5

Effect of petrol and diesel oil contamination on magnesium (Mg) content in aerial parts of plants ($g \cdot kg^{-1}$ d.m.)

Dose of Pet or DO ($cm^3 \cdot kg^{-1}$ of soil)	Contamination							
	petrol (Pet)				diesel oil (DO)			
	kind of substance neutralizing effect of Pet and DO							
	without additions	compost	bentonite	CaO	without additions	compost	bentonite	CaO
Spring oilseed rape (*Brassica napus* var. *oleifera*) – main crop								
0	2.32	2.64	2.17	3.14	2.32	2.64	2.17	3.14
2.5	2.60	2.55	2.92	2.69	4.48	4.15	2.73	2.64
5.0	2.60	2.93	3.06	2.53	4.70	4.27	3.23	2.95
10.0	3.17	2.83	3.28	2.54	3.47	4.84	4.19	4.00
r	0.969**	0.639*	0.874**	-0.795**	0.280	0.878**	0.999**	0.758**
LSD	*a* – 0.04 **, *b* – 0.06 **, c – 0.06 **, *a·b* – 0.08 **, *a·c* – 0.08 **, *b·c* – 0.12 **, a·b·c – 0.16 **							
Oats (*Avena sativa* L.) – aftercrop								
0	3.69	2.95	2.76	2.93	3.69	2.95	2.76	2.93
2.5	3.45	3.25	2.88	3.48	3.18	3.58	2.73	2.98
5.0	3.50	3.91	2.88	3.23	3.60	3.52	3.11	2.86
10.0	2.86	2.73	2.73	3.10	3.17	2.97	2.86	2.97
r	-0.944**	-0.183	-0.315	0.038	-0.577	-0.163	0.379	0.125
LSD	*a* – n.s., *b* – 0.07 **, *c* – 0.07 **, *a·b* – 0.11 **, *a·c* – 0.11 **, *b·c* – 0.15 **, *a·b·c* – 0.21 **							

LSD for: *a* – petroleum substance, *b* – petroleum substance dose, *c* – neutralizing substance
**significant at p=0.01, *significant at p=0.05, n.s. – non-significant, *r* – correlation coefficient

These results are confirmed by the literature on the effect of petroleum-derived compounds on plants (IWANOW et al. 1994, AMADI et al. 1996, WYSZKOWSKI et al. 2004). The negative effect of such pollutants is a product of the behaviour of petroleum-derived compounds in soil, where they block air spaces that allow air and water to enter soil layers, which causes soil lumping and deteriorates physical, chemical and biological properties of soil. The organic carbon to nitrogen ratio in soil contaminated with petroleum-derived products is typically unfavorable. Therefore, reactions of mineral and organic nitrogen compounds in soil are inhibited. The rate of ammonification and nitrification decreases (IWANOW et al. 1994, AMADI et al. 1996) while bacteria and fungi develop intensively and consume macroelements. The content of plant available macroelements in soil decreases (XU, JOHNSON 1997). Such a development has been confirmed in the present study and in earlier re-

Table 6

Person's simple correlation coefficient between content of macroelements in plants and yield and some properties of soil

Variable	Content in plants									
	spring oilseed rape (*Brassica napus var. oleifera*)					oats (*Avena sativa* L.)				
	P	K	Na	Ca	Mg	P	K	Na	Ca	Mg
Plants										
Yield	-0.075	-0.508**	0.009	-0.654**	-0.726**	-0.275*	0.408**	-0.432**	-0.081	0.182
N	0.730**	0.530**	0.276*	0.127	0.148	0.491**	0.113	0.413**	-0.283*	0.005
P	X	0.646**	0.456**	0.111	0.173	X	0.062	0.545**	-0.290*	-0.110
K	0.646**	X	0.227	0.341**	0.527**	0.062	X	-0.444**	0.012	-0.125
Na	0.456**	0.227	X	-0.019	0.010	0.545**	-0.444**	X	-0.559**	-0.380**
Ca	0.111	0.341**	-0.019	X	0.840**	-0.290*	0.012	-0.559**	X	0.437**
Mg	0.173	0.527**	0.010	0.840**	X	-0.110	-0.125	-0.380**	0.437**	X
Soil										
pH KCl	0.061	-0.103	0.329**	0.155	-0.198	0.089	-0.273*	0.424**	0.196	-0.397**
HA	-0.171	0.070	-0.425**	-0.156	0.179	-0.116	0.361**	-0.582**	-0.041	0.445**
ECB	0.233	0.028	0.435**	0.087	-0.139	-0.290*	-0.182	0.274*	-0.061	-0.235
CEC	0.194	0.061	0.317*	0.034	-0.084	-0.378**	-0.071	0.093	-0.086	-0.101
BS	0.258*	-0.055	0.445**	0.158	-0.198	-0.040	-0.390**	0.541**	0.009	-0.401*
$N\text{-}NO_3$	-0.065	-0.068	-0.087	-0.249*	-0.005	0.037	0.538**	-0.259*	-0.244	0.085
$N\text{-}NH_4$	-0.045	0.195	-0.130	0.496**	0.568**	0.376**	0.710**	0.012	-0.146	-0.294*
C_{org}	0.014	0.419**	-0.230	0.569**	0.615**	-0.020	0.012	-0.274*	0.132	0.407**
$P_{available}$	0.132	0.019	0.103	0.203	0.085	0.107	-0.154	0.194	0.291*	-0.157
$K_{available}$	0.031	0.393**	0.173	0.600**	0.675	0.433**	0.640**	0.087	-0.347**	-0.260*
$Mg_{available}$	0.036	0.144	0.553**	-0.024	0.139	0.426**	0.571**	0.307*	-0.667**	-0.277*

**significant at p=0.01, *significant at p=0.05

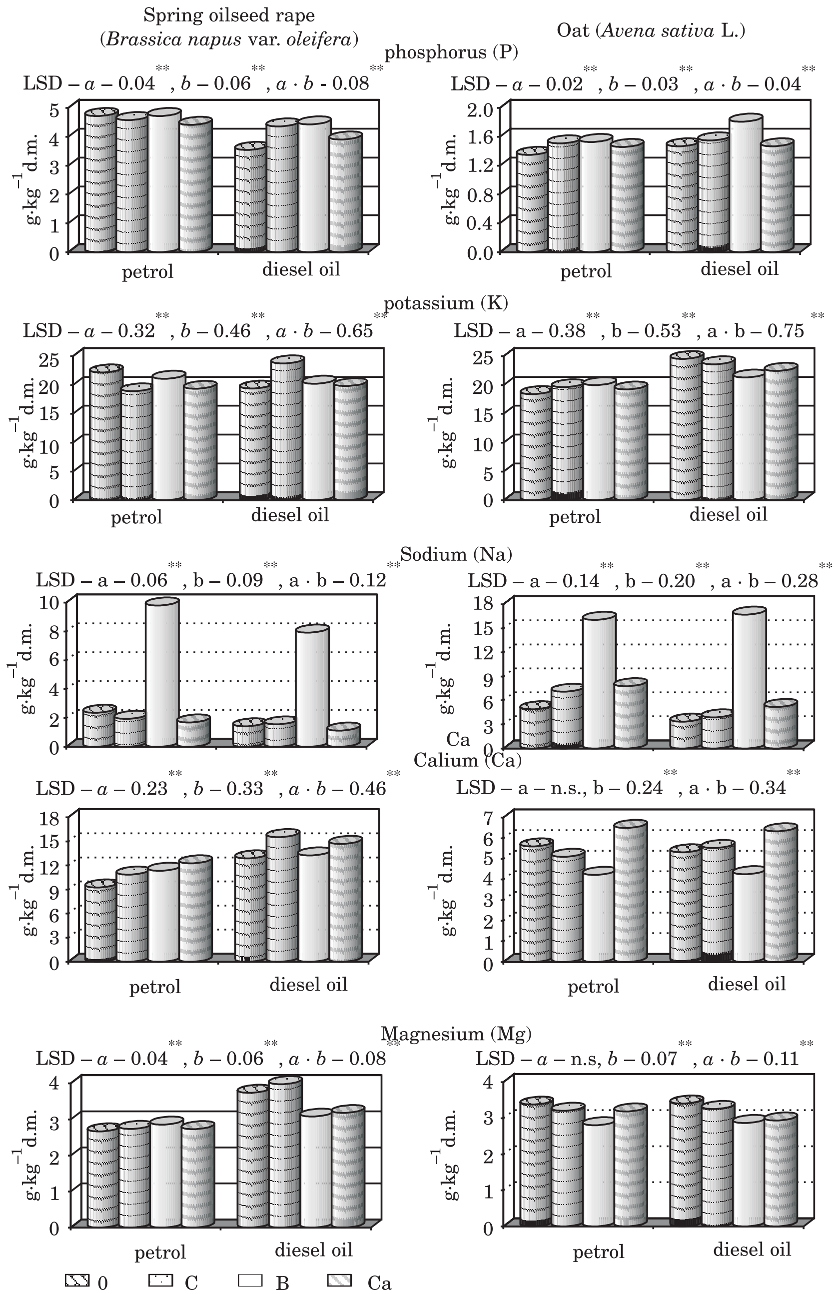

Fig. 1. Comparison of the effect of a neutralizing substances on the content of some macroelements of plants, in g·kg^{-1} d.m. (average for series):
0 – without substances, C – with compost, B – with bentonite, Ca – with calcium oxide
LSD for: a – kind of a petroleum substances, b – addition of a neutralizing substances
**significant for p=0.01, *significant for p=0.05, n.s. non-significant

search (Wyszkowski, Wyszkowska 2005), in which limited content of macroelements in oats from soil contaminated by high doses of diesel oil was demonstrated. Another experiment reported by Wyszkowski and Wyszkowska (2005) indicates correlation between the enzymatic activity of soil and the content of nitrogen and phosphorus in plants. However, this correlation was strongly modified by the presence of diesel oil, nitrogen and sawdust in soil.

The effect of petrol and diesel oil on macroelements in plants is determined by many factors, of which plant variety seems to be the most important. In the study by Dimitrov and Mitowa (1998) only 3 out of 7 experimental plant species contained modified levels of some macroelements when cultivated on diesel oil-contaminated soil in comparison to uncontaminated soil. According to Wyszkowski and Wyszkowska (2005), content of most macroelements in aerial parts of maize on diesel oil-contaminated soil is likely to increase.

Role of compost, bentonite and calcium oxide in modification of the influence petroleum-derived products on plants' chemical composition

A very important factor which modified content of macroelements in plants was the addition of compost, bentonite or calcium oxide (Tables 1-4, Figure 1). On average, irrespective of the degree of soil contamination, all components introduced to soil in order to alleviate possible negative effects of petroleum-derived products significantly modified the content of macroelements in aerial parts of both plants. Obviously, the effect of the neutralizing substances varied depending on the dose of petrol and diesel oil added to soil. The effect of a neutralizing substance (compost, bentonite and calcium oxide) on the content of the majority of macroelements was stronger in spring oilseed rape than in oats. Compost, bentonite or CaO more strongly contributed to increasing the content of sodium, calcium and magnesium than phosphorus and potassium in plants. Bentonite was the effective in increasing sodium in aerial parts of both plants, especially spring oilseed rape. In the bentonite-treated series, the highest increase in sodium content in spring oilseed rape (*ca* 9-fold in petrol objects and *ca* 7-fold in diesel oil objects) was observed in either uncontaminated objects or polluted with the highest doses of petrol and diesel oil. Calcium oxide and, to a lesser degree bentonite (only in spring oilseed rape), increased the content of calcium in plants. As for magnesium, the correlation was reverse. The results obtained after the application of compost were less consistent.

The application of compost and other substances to soil usually have a positive effect on soil properties, plants' growth and chemical composition (Vouillamoz, Milke 2001). Compact soils rich in humus are far more tolerant to degradation than sandy soils (Wyszkowski et al. 2004). Organic substance improves absorbance of petroleum products and has influence on the biological life of soil (Małachowska-Jutsz et al. 1997) and consequently on plants.

Liming improves properties of soil. Bentonite added to soil forms a compact barrier, which prevents petroleum products from reaching deeper horizons of the soil profile. Moreover, content of elements available to plants rise, which is of importance for plant growth and development (WYSZKOWSKI et al. 2004, WYSZKOWSKI, WYSZKOWSKA 2005).

CONCLUSIONS

1. Irrespective of the application of compost, bentonite or calcium oxide, the highest doses of petrol and especially diesel oil decreased the content of most macroelements in spring oilseed rape and, to a smaller degree, in oats.

2. Enrichment of soil contaminated with petroleum-derived products with organic matter, bentonite or calcium oxide improved chemical composition of plants, which was demonstrated as increased content of some macroelements in plants. These neutralizing substances added to soil increased its tolerance to eco-toxic effects of petrol and diesel oil.

3. Enrichment of soil with compost, bentonite or calcium oxide modified the content of macroelements in plant, mainly sodium when bentonite was used.

4. Some strong and significant correlations between the content of macroelements in spring oilseed rape and oats versus plants yield and content other elements in plants, as well as some properties of soil were observed.

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